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Fighting *Staphylococcus aureus* biofilms with monoclonal antibodies

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Abstract

Staphylococcus aureus (*S. aureus)* is a notorious pathogen and one of the most frequent causes of biofilm-related infections. The treatment of *S. aureus* biofilms is hampered by the ability of the biofilm structure to shield bacteria from antibiotics as well as the host's immune system. Therefore, new preventive and/or therapeutic interventions, including the use of antibody-based approaches, are urgently required. In this review, we describe the mechanisms by which anti-*S. aureus* antibodies can help in combatting biofilms, including an up-to-date overview of monoclonal antibodies currently in clinical trials. Moreover, we highlight ongoing efforts in passive vaccination against *S. aureus* biofilm infections, with special emphasis on promising targets, and finally indicate the direction into which future research could be heading.

Keywords

Staphylococcus aureus; infection; biofilm; monoclonal antibodies; vaccine; passive immunization

1. Clinical significance of *S. aureus* biofilm-associated infections

The Gram-positive pathobiont *Staphylococcus aureus* (*S. aureus*) is one of the most frequent causes of nosocomial infections, and there is no vaccine available yet. *S. aureus* infections are highly diverse, ranging from acute diseases, such as bacteremia and skin abscesses to severe chronic infections that are often associated with biofilms [1]. Due to an arsenal of **adhesins** (see Glossary), *S. aureus* can attach to and persist on host tissues (e.g. heart valves and bones) as well as implanted materials (e.g. catheters, prosthetic joints and pace makers), and cause diseases such as endocarditis, and osteomyelitis [1–3]. On the other hand, about 20% of the human population is persistently colonized in the anterior nares and other body

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sites such as the intestine, while the remainder carry the bacteria intermittently [4]. In most cases, colonization is asymptomatic, but it can also lead to endogenous infections [5].

Over the past decades, the steady increase in the use of medical implants has been accompanied by a rise in infection risk. Indeed, implant or device-associated infections are important complications associated with the use of biomaterials [2,6], and account for one quarter of all healthcare-associated infections in the USA [7]. Among their deleterious consequences are failure of prosthetic devices, implant replacement with its associated risk of clinical complications, and chronic and/or relapsing diseases [2,8]. Staphylococci, including *S. aureus, S. epidermidis* (Box 1) and other coagulase-negative staphylococci (CoNS) are the main culprits of foreign body-associated infections, accounting together for an estimated 80% of all infections [2,9]. The diagnosis and targeted therapy of implant infections is often problematic, because they are frequently subclinical and culture-negative.

Biofilm formation is an important virulence mechanism of many bacterial pathogens. A biofilm is defined as a sessile microbial community embedded within an amorphous slimy material [2]. Biofilm formation enables growth on natural and foreign surfaces, and shields bacteria from antibacterial therapies as well as the host immune system, often leading to persistent infections unresponsive to antibiotic therapy [2]. In addition to the matrix representing a penetration barrier for many antimicrobial agents, the efficacy of most antibiotics is reduced against biofilms, because cells in a biofilm are in a state of reduced metabolism [10,11], whereas most antibiotics target active cell processes, such as cell wall formation, translation or transcription [12]. Consequently, there is an immense medical need to develop innovative preventive and/or therapeutic interventions, including anti-infective biomaterials, biofilm-active antibiotics, and biofilm matrix-degrading enzymes [13]. Another appealing measure to prevent biofilm formation and/or treat established biofilms is the use of monoclonal antibodies targeting the invasive pathogen, which is the focus of this review. After describing aspects of *S. aureus* biofilm formation, the antibacterial antibody response in *S. aureus* biofilm infections as well as techniques to generate monoclonal antibodies, we provide an update on preclinical as well as clinical studies on monoclonal antibodies against *S. aureus* biofilm-associated infections and outline critical aspects for the development of a successful anti-biofilm vaccine.

2. Staphylococcal biofilm stages and composition

In order to develop protective antibody-based therapies, it is essential to gain an in-depth understanding of the process of biofilm formation as well as its composition, since gene and protein expression differ greatly between the planktonic and biofilm modes of bacterial growth [14,15]. In the past, proteomic analyses were usually based on examining intracellular proteomes of laboratory isolates in static biofilms. However, more recent studies used flow chamber systems or even analyzed biofilms from animal infection models, which may reflect the human clinical situation more closely and hence reveal novel biofilm-associated targets [14,16–19].

Biofilm formation in staphylococci has been described as a process comprising at least three main stages: (i) bacterial attachment to a surface, (ii) biofilm formation and maturation, and

(iii) biofilm detachment / dispersal (Figure 1, Key Figure) [20]. Staphylococcal agglomerations that are not attached to a surface are also occasionally regarded as biofilms [21]; in those cases, intercellular aggregation substitutes for the initial adhesion step.

Attachment of bacteria to abiotic plastic surfaces of indwelling medical devices may happen via hydrophobic attraction. However, soon after insertion, human matrix proteins cover the device surfaces, and thus, initial attachment *in vivo* proceeds mainly via the interaction of staphylococcal surface binding proteins with human extracellular matrix [20]. Many of the former belong to the “microbial surface components recognizing adhesive matrix molecule” (MSCRAMM) family [22]. MSCRAMMs (discussed in detail in Chapter 5) are anchored to the cell wall via the enzyme sortase and contain cell wall-spanning domains that end with an exposed domain binding to human matrix proteins (Figure 1). Overall, there is pronounced redundancy among the MSCRAMMs, reflecting their key role in bacterial colonization and survival in the host [22].

The biofilm formation / maturation phase, in addition to bacterial growth, is characterized by the secretion of biofilm matrix components and the creation of a three-dimensional biofilm structure. The composition of biofilm matrix is heterogeneous, comprising proteins, extracellular DNA (eDNA) and polysaccharides (discussed in detail in Chapter 5). Several secreted proteins have been implicated in biofilm formation, many of them are surface binding proteins, whose contribution to the initial adhesion versus subsequent phases of biofilm development is often hard to discern. In contrast, the *S. epidermidis* accumulation-associated protein Aap and its *S. aureus* homologue SasG, appear to have a very specific biofilm matrix function, forming polymeric fibrils that link together cells in a biofilm [23–25].

Another biofilm-characteristic component is the cell surface associated exopolysaccharide PNAG (ß-1,6-poly-*N*-acetylglucosamine, also called polysaccharide intercellular adhesin, PIA) [26]. PNAG is not omnipresent in staphylococcal biofilm-forming isolates [27], but its production supports cell to cell adhesion, leading to more robust biofilms [28,29]. PNAG’s cationic nature facilitates bacterial attachment to host cell surfaces [30], which is possibly mediated by negatively charged molecules such as teichoic acids and eDNA that is released by dying cells.

Biofilms do not grow as undifferentiated “bricks”, but contain channels that are deemed important for nutrient delivery to all layers of a biofilm. Enzymatic digestion of biofilm matrix molecules, such as eDNA and proteins by nucleases and proteases, respectively, has been implicated in channel formation [1]. However, no enzyme capable of degrading PNAG has so far been identified in staphylococci. Moreover, phenol-soluble modulins (PSMs) are amphipathic and surfactant-like peptides that structure biofilms independently of biofilm matrix composition, most likely by disrupting hydrophobic as well as hydrophilic interactions between biofilm matrix molecules [31,32].

Detachment of cells or cell clusters from a biofilm can be triggered solely by mechanical shear forces as encountered in the blood stream. However, this process, which is crucial for the systemic dissemination of a biofilm-associated infection, can also be facilitated by

pronounced activity of biofilm-structuring factors. For instance, PSMs disrupt interactions of biofilm matrix molecules, such as PNAG, with each other *in vivo*, contributing to biofilm dispersal [31,33].

3. Antibacterial antibody response in *S. aureus* biofilm infections

How the innate and adaptive immune systems react to biofilms and what type of immune response is protective is still not well understood. Biofilm infections trigger an inflammatory response, as reflected by the infiltration and activation of phagocytes at the site of infection, and the release pro-inflammatory cytokines, promoting a Th1/Th17 response and the production of antibodies, predominantly of the human IgG1 subclass [34–36]. While neutrophils are capable of infiltrating the biofilm and efficiently phagocytose enclosed cells, this defense mechanism is less effective in mature biofilms [37]. This exemplifies the inefficiency of the induced host response in clearing a persistent biofilm infection [37,38].

Similarly, the protective potential of antibodies in biofilm infections is not well defined. *S. aureus* infection stimulates the production of specific antibodies against a broad range of surface and secreted staphylococcal proteins, but these generally do not prevent a re-infection with this notorious pathogen [39]. However, antibody profiling in sepsis patients at the time of diagnosis showed that high antibody titers might confer protection from an adverse outcome [40]. This implies that the immunological “starting position” is important for disease outcome, a fact that is encouraging for efforts in vaccine development. For biofilm infections, clinical data are scarce, but suggest that biofilms also trigger or boost an antibody response against a broad range of *S. aureus* antigens: adhesins and cell wall-modifying enzymes, biofilm matrix components, toxins and immune evasion factors (discussed in detail in Chapter 5) [41–43].

In line with these patient data, animal experiments indicate that boosting the antibody response by active or passive vaccination prevents or at least reduces the severity of biofilm-associated *S. aureus* infections [18,35,44]. For example, in a murine model of mesh-associated biofilm infection, a vaccination approach using biofilm matrix exoproteins significantly reduced the number of bacterial cells inside a biofilm and on the surrounding tissue [18]. Another multivalent *S. aureus* vaccine comprising four cell wall-associated proteins prevented the formation of biofilm-mediated osteomyelitis in the majority of the treated animals when combined with an antimicrobial therapy [44]. Hence, animal data suggest that antibodies can contribute to biofilm prevention and clearance.

Anti- *S. aureus* antibodies can penetrate the biofilm matrix [45,46], and interfere with all three stages of biofilm formation. Initial attachment can be prevented by targeting surface-bound or soluble adhesins (Figure 2). Biofilm maturation is disturbed by blocking surface proteins involved in cell-to-cell adhesion, and biofilm dispersal is enhanced by targeting matrix-stabilizing proteins. Moreover, high affinity IgA and IgG antibodies can neutralize secreted bacterial factors (e.g. toxins, enzymes, immune evasion molecules). Finally, surface-bound antibodies can enhance biofilm elimination by neutrophils and macrophages, either via antibody-binding Fc receptors or by inducing complement activation and C3b deposition on the bacterial surface (Figure 2) [18,47,48]. In conclusion, antibodies can

potentially interfere with biofilm formation and/or promote dispersal of established biofilms by several mechanisms. However, since the natural antibody response in many cases seems to be insufficient to eliminate established biofilms, boosting the antibody response by active or passive vaccination seems a promising approach to reduce the severity of biofilm-associated *S. aureus* infections.

4. Generation of monoclonal antibodies

Monoclonal antibodies are superior to polyclonal sera in studying anti-biofilm activities, since they allow for molecular interaction studies and can potentially be applied in human patients. Over the past decade, technical advances have been made in the production and modification of monoclonal antibodies. Traditionally, monoclonal antibodies were generated using the hybridoma technology (Figure 3A) resulting in murine antibodies, which can, however, have severe side effects if introduced into the human host [49,50].

Within less than a decade after the first monoclonal antibody was described, two approaches were developed to reduce antigenicity and enhance antibody-mediated effector functions. The recombinant attachment of a murine antigen-specific variable region to a human constant region resulted in the rise of chimeric antibodies [51]. Almost concurrently, the first humanized antibody was generated by transfering the murine complementarity-determining regions (CDRs) into a human antibody sequence. Especially the latter approach reduced the murine proportion and hence the immunogenicity of the antibody [52]. Both methods enabled the selection of the desired human constant region, which defines the antibody class and hence antibody-mediated effector functions. Nowadays it is possible to generate chimeric and humanized antibodies by combining the use of transgenic mice expressing chimeric or CDR-drafted antibodies with the hybridoma technology.

The four most common techniques for the generation of numerous fully human monoclonal antibodies are phage display libraries, B cell cultures, B cell immortalization using Epstein-Barr virus (EBV) as well as cloning of antibodies from single antigen-specific B cells (Figure 3B-E). While phage display libraries are generated by random combination of immunoglobulin heavy and light chains, the other three methods are based on antigen-specific B cells from exposed donors and thus reflect the physiological antibody response [53,54]. These methods can also be elegantly combined. For example, instable hybridomas and EBV-transformed cell lines can be rescued by cloning the antibody-coding sequences into an expression system.

Using antibody engineering, it is also possible to tailor the affinity and effector functions of antibodies to their application. For example, monoclonal antibodies can be genetically modified to increase antigen affinity and antibody half-life or to increase/decrease affinity towards Fc receptors [55,56]. Moreover, two antibodies can be combined to create bispecific antibodies by linking Fab fragments of two different specificities [57,58], or used as a shuttle to target antimicrobial drugs directly to the *S. aureus* cells [59–61]. This tool box for antibody engineering promises to be very helpful in designing protective anti-*S. aureus* monoclonal antibodies in the future.

5. Preclinical studies on antibodies targeting *S. aureus* biofilms

Despite the huge clinical impact of biofilm infections, research on antibodies targeting *S. aureus* infections often ignores biofilms during antigen selection and preclinical antibody testing. Nevertheless, there are several interesting *S. aureus* vaccine candidates with promising results in pre-clinical studies that we would like to highlight in this chapter, including adhesins, cell-wall modifying enzymes, surface glycopolymers, biofilm matrix components, and toxins (Figure 1).

Adhesins

Staphylococcal adhesins, including MSCRAMMs, are one of the most studied targets for antibody-based therapies [22]. Antibodies against adhesins exert their action via two mechanisms (Figure 2): (i) preventing the initial microbial adherence to abiotic as well as biotic surfaces [62–64], and (ii) coating the bacterial surface, thereby facilitating the clearance of an organism through opsonophagocytic bacterial killing [65,66]. In the context of biofilm vaccine development, interesting candidates are clumping factor (Clf) A and B, and the fibronectin-binding proteins (FnBPA and B) as these cell-wall associated proteins are involved in biofilm formation and are widely distributed among the *S. aureus* clinical isolates, while the collagen-binding protein (Cna), biofilm-associated protein (Bap), as well as the *S. aureus* surface protein C (SasC) and SasG are present only in a subset of isolates [67].

The MSCRAMM ClfA promotes bacterial binding to fibrinogen. ClfA plays an important role in the colonization of implanted biomaterials or damaged endothelial surfaces at the site of endovascular infections [22,68]. Over the past 15 years, several monoclonal antibodies against ClfA were shown to block biofilm formation *in vitro* (Table 1) [62,66,69,70]. In animal infection models anti-ClfA monoclonal antibodies protected from biofilm (e.g. IE) and non-biofilm-associated (e.g. sepsis) infections [69,70]. In contrast, an anti-ClfA monoclonal antibody alone had only a moderate effect in a murine hematogenous implant infection, but in combination with antibodies against alpha toxin (Hla) it effectively inhibited biofilm formation both *in vitro* and *in vivo* [62]. Moreover, a humanized anti-ClfA monoclonal antibody (tefibazumab) conferred full protection against infective endocarditis (IE) in rabbits when applied prophylactically [69].

FnPBs recognize fibronectin, fibrinogen and elastin, and promote intercellular accumulation and biofilm development (Table 1) [22]. Antibodies against FnBP inhibit *S. aureus* biofilm formation *in vitro* and partially protected mice against endocarditis following sepsis [63,64].

Cell wall-modifying enzymes

The surface-associated murein hydrolase autolysin, Atl is a bifunctional enzyme that undergoes proteolytic cleavage to yield two cell wall-active enzymes, an amidase (Amd) and a glucosaminidase (Gmd). Both enyzmes are involved in bacterial cell separation after cell division, host extracellular matrix adhesion and biofilm formation (Table 1) [71]. Polyclonal antibodies to Amd and Gmd inhibit biofilm formation and enhance opsonophagocytosis

[71,72]. In addition, a monoclonal antibody against Gmd (1C11) reduced infection severity in a murine model of implant-associated osteomyelitis [73].

Glycopolymers

Staphylococcal cells are decorated with glycopolymers, including wall teichoic acids (WTA), peptidoglycan, lipoteichoic acids (LTA), and capsular polysaccharides (CP). These surface glycopolymers are recognized by serum antibodies and a variety of pattern recognition molecules, including mannose-binding lectin. Anti-WTA antibodies facilitate complement C3 deposition via the classical pathway as well as opsonophagocytosis of laboratory and clinical *S. aureus* isolates by neutrophils (Table 1) [74,75]. Although a human monoclonal anti-WTA antibody was ineffective in preventing *S. aureus* infection in an intravenous mouse infection model, it showed promising *in vivo* results when conjugated to an antibiotic [61]. Further human monoclonal antibodies targeting WTA are currently characterized [76]. However, to the best of our knowledge, anti-WTA antibodies have never been tested in biofilm-related infection models. Antibodies against the capsular polysaccharides promote opsonophagocytosis but yielded contradictory results when tested in a rat endocarditis models. While rabbit polyclonal antibodies conferred partial protection, murine antibodies were not protective [77–79].

Biofilm matrix

The biofilm matrix has been recently brought into the focus of anti-biofilm vaccine research. This is in part due to the widely conserved nature of some of its components, making those components suitable conserved vaccine candidates for protection against various human pathogens.

PNAG has been extensively evaluated as a potential vaccine candidate in relation to biofilm-associated infections (Table 1). In contrast to many *S. aureus*-specific biofilm factors, it is expressed among a variety of bacteria, fungi and protozoa [80,81]. For instance, the immunological cross-reactivity of an opsonic antibody against *S. aureus* PNAG and *Escherichia coli* polyglucosamine has led scientists to investigate the possibility of developing a vaccine against both pathogens [80]. Several studies have highlighted the superiority of deacetylated PNAG (dPNAG) to PNAG in terms of immunogenicity and protection in animal models [80,82]. Anti-dPNAG immune sera provided efficient protection in a murine intraperitoneal [80,82], as well as a bacteremia model [82]. More interestingly, the human IgG1 monoclonal antibody F598 (which binds both PNAG and dPNAG) has opsonic and protective activities against multiple microbial pathogens *in vivo* [65,81] and is currently undergoing preclinical and clinical assessments as a broad-spectrum antimicrobial therapeutic [83].

Bacterial DNA-binding proteins (DNABII family) have conserved homologs in a wide variety of bacterial species and are involved in a number of biofilm-associated infections [84,85]. They serve as adapter proteins for eDNA strands and hence stabilize the biofilm matrix (Figure 1; Table 1) [86]. Loss of these scaffolding proteins, for instance by neutralization with specific antibodies, causes dispersal of the biofilm. The released bacteria

regain their susceptibility to killing by antibiotics and are more easily cleared by phagocytes [86,87].

Recently, Estellés et al. generated a native human monoclonal antibody (TRL1068) recognizing a DNABII epitope conserved across a range of Gram-positive and Gram-negative bacterial species [84]. TRL1068 showed anti-biofilm efficacy in an *in vitro* biofilm assay as well as in a murine infectious implant model, and a catheter-related biofilm infection model in rats [84,85]. However, as this antibody promotes biofilm dispersal, it is essential to eliminate the released bacteria to prevent subsequent dissemination to distant organs. Therefore, TRL1068 was proposed as a clinical candidate for the treatment of implant-associated infections in combination with standard-of-care antibiotics (Table 1) [84,85].

Toxins

Proteomic studies demonstrated that several pore-forming toxins (e.g. Hla, LukAB, and γ hemolysin (HlgAB)), and immune evasion molecules (e.g. SCIN, and CHIPS) are produced within a biofilm *in vitro* and *in vivo*, some even in higher amounts than in planktonic cultures, whereas others, including the immune evasion protein A, are down-regulated [14,17,88]. The pore-forming toxins Hla, LukAB and HlgAB lyse a range of host immune cells, including T cells, monocytes and neutrophils [89], thereby torpedoing the anti-biofilm immune response. Neutralizing these toxins by monoclonal antibodies may enhance host defenses and facilitate clearance of planktonic and biofilm cells (Figure 1).

Apart from destroying immune cells, Hla promotes biofilm formation *in vitro*, as well as *in vivo* by disrupting the host epithelium, providing nutrients for bacterial survival through promoting host cell lysis, and facilitating bacterial cell-to-cell interactions [90,91]. The human monoclonal anti-AT neutralizing antibody (MEDI4893) sterically inhibits binding of Hla to its cellular receptor ADAM10, effectively blocking pore formation [92]. It successfully abrogated *ex vivo* biofilm formation on porcine vaginal mucosa explants [91]. Considering that prophylactic treatment with MEDI4893 in a mouse model of *S. aureus* wound infection also promotes wound healing [93], this suggests that neutralization of Hla may be useful in biofilm-related *S. aureus* wound infections. MEDI4893 has been extensively tested in various biofilm and non-biofilm infection models (Table 1) [62,91,93].

The pore-forming toxin leukocidin A/B (LukAB) kills professional phagocytic cells, and together with AT facilitates the persistence of staphylococcal biofilms [94]. Badarau et al. first reported on the discovery of a highly potent neutralizing human IgG1 monoclonal antibody against LukAB (ASN-2), with a high affinity antibody binding site on the LukAB dimer [95]. In 2017, Thomsen et al. reported on three potently neutralizing naturally occurring LukAB-specific human monoclonal antibodies, which reduced the bacterial load in murine sepsis model (Table 1) [96].

Quorum-sensing blocking monoclonal antibodies

Targeting quorum sensing, i.e. bacterial cell density-dependent gene regulation, is a frequently promoted antivirulence strategy [97]. In *S. aureus* and other staphylococci, the quorum-sensing system Agr controls virtually all known virulence factors, such as toxins

and secreted degradative enzymes [98]. In an exceptionally strict fashion, Agr controls thePSMs [99], which – as previously mentioned – trigger biofilm structuring and detachment [100]. Owing to this control, interfering with Agr quorum sensing results in the formation of thick undifferentiated biofilms [101]. Another less well characterized potential quorum-sensing system, LuxS, controls exopolysaccharide synthesis in a negative fashion [102,103]. Thus, interfering with quorum-sensing in staphylococci by the use of monoclonal antibodies or any other means does not represent a promising/efficient anti-biofilm strategy.

In summary, several *S. aureus* vaccine candidates, including adhesins, cell-wall modifying enzymes, biofilm matrix components and toxins, showed promising results in pre-clinical studies. To combat biofilm-related infections, future vaccination studies should aim at identifying and testing bacterial target structures expressed by both planktonic and biofilm cells, for instance using proteomic approaches.

6. Clinical trials on antibodies targeting *S. aureus*

Several of the above described targets, including ClfA, CP5 and 8, PNAG, Hla and HlgAB (Table 2), have been tested as passive vaccines in clinical phase II and/or III trials. However, none of them improved the clinical outcome in the treated patient cohorts. For instance, the anti-ClfA monoclonal antibody tefibazumab failed to achieve statistically significant improvement of clinical outcome in bacteremia and cystic fibrosis patients [104]. Similarly, polyclonal antiserum against CP5 and CP8 (AltaStaph), as well as a monoclonal antibody against LTA (Pagibaximab) failed in phase II and III trials, respectively (Table 2, Box 1) [105–108]. Moreover, a phase IIa study on using an anti-PNAG monoclonal antibody in ventilated intensive care unit (ICU) patients was terminated.

This failure of trials using surface-directed monoclonal antibodies against adhesins and surface glyocopolymers forced *S. aureus* researchers to revisit *S. aureus* pathogenesis and potential correlates of protection. One lesson learned is that targeting a single adhesin is prone to failure due to the high functional redundancy of these proteins. For instance, there are at least five fibrinogen-binding proteins in *S. aureus* [22]. Moreover, it has been suggested that adverse effects could be caused by antibody-induced agglutination, since large aggregates of bacteria in the blood may not be cleared by the host and could become trapped in various tissues, particularly in the lungs [109,110]. Finally, in contrast to other pathogens, opsonophagocytosis may not be the most important mechanism of protection, since this species produces a whole arsenal of toxins and immune evasion proteins that are decisive for pathogenesis. In consequence, current research and clinical trials are focusing on poreforming toxins as targets for an antibody-based therapy (Table 2) [95,111,112]. MedImmune as well as Aridis Pharmaceuticals are testing human anti-Hla antibodies for prevention of *S. aureus* pneumonia [111].

Apart from a shift towards toxins, there is now a trend towards multivalent vaccines in order to combat the multifactorial nature of *S. aureus* pathogenesis [112,113]. For instance, Arsanis Biosciences has tested a combination of two human monoclonal antibodies (ASN-1 targeting Hla and four other bi-component leukocidins; ASN-2 targeting LukAB) in the ASN100 phase II clinical trial for the prevention of *S. aureus* pneumonia (Table 2) [95,112].

The trial was however recently halted due to insufficient efficacy. Another approach involves the use of monoclonal antibodies as means of targeted delivery of antimicrobials. For instance, an antibody-antibiotic conjugate (AAC) specifically binding wall teichoic acid is currently used in a phase Ib clinical trial targeting *S. aureus* bacteremia patients [114,115] (Table 2).

7. Concluding Remarks and Future Perspectives

Although biofilm infections have been recognized as an important mediator of chronic infection associated with high morbidity and mortality, vaccine research has seemingly overlooked biofilms with regard to discovery and efficacy studies. A better understanding of the immune response against biofilms, and of how biofilms manipulate this response, is therefore essential for the development of protective staphylococcal vaccines (see Outstanding Questions). Nevertheless, in the reasonably near future, the identification and testing of new combinations of monoclonal antibodies, which are effective against planktonic as well as biofilm cells in a broad range of disease settings, will hopefully achieve more success than past attempts.

Ideally, the following factors should be considered while selecting potential biofilm-related antigens: (i) prevalence in clinical isolates [67], (ii) antigenic variability of the target protein [66,116,117], (iii) expression profiling of proteins within the biofilm *in vivo* [14,16], (iv) its relevance in many different staphylococcal diseases, (v) immunological relevance, i.e. accessibility to antibodies within the biofilm matrix, and (vi) ability to induce not only a strong but the correct (i.e. protective) type of immune response (governed by the right choice of adjuvant / route of antigen application) [118].

In order to meet all or most of these criteria, multivalent vaccines are the only strategy of choice for active as well as passive immunization [18,44,44,66]. The most effective therapeutic approach for the biofilm lifestyle will likely require a combinatorial approach of bactericidal and immunostimulatory treatments. It may be an unrealistic goal to achieve a complete clearance of *S. aureus* from our body, bearing in mind that the microorganism is part of the human normal microbiota and an expert in evading host immune defense, but rather clinical protection to reduce the severity of staphylococcal infections and prevent chronification.

Recapitulating the unsuccessful clinical trials for a passive *S. aureus* vaccine, several hurdles can be named: (i) the multiplicity and redundancy of *S. aureus* virulence factors which challenges the selection of protective antigens, (ii) the production of numerous immune evasion factors, including protein A (iii), a lack of knowledge about the nature of protective immunity against *S. aureus* infection, and (iv) a lack of transition from animal models to human studies [109,119,120]. One explanation for a lack of transition could be the use naive animals, whereas humans are immunologically primed against *S. aureus.* This may explain why huge effects of different passive or active vaccination strategies in animals cannot be reproduced in humans. If these points are considered in antigen selection processes and subsequent preclinical tests, we will hopefully be more successful in the near future in developing a protective passive vaccine against this notorious pathogen.

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Glossary

Active vaccination:
immunization with an antigen to provoke adaptive immunity. It usually induces long-lasting and robust protective immune memory, but requires several weeks to become fully effective.

Adhesins:
bacterial cell surface proteins that enable them to bind to the surfaces of host cells or the extracellular matrix.

Antibody:
also known as immunoglobulin (Ig), is secreted by B cells upon antigen contact. It is highly specific and binds its target structure, the antigen, with very high affinity. While each B cell produces identical antibodies, an individual can produce a total number of at least 10^7 antibody specificities, enabling the immune system to respond to wide range of antigens. Structurally, antibodies are heterodimeric proteins composed of two heavy and two light chains which are linked by disulfide bonds.

Chimeric antibody:
antibody whose constituent parts are derived from different species, mostly human and murine. The replacement of the murine with a human Fc part allows chimeric antibodies to efficiently interact with the human immune system and reduces the risk of an adverse immune response to the applied monoclonal antibodies.

Human antibody:
antibody that is composed of fully human antibody heavy and light chains.

Humanized antibody:
antibody, where the mouse antigen binding regions (= hypervariable loops) are genetically engineered into otherwise human antibodies.

Hybridomas:
hybrid cell lines formed by fusing a myeloma cell (no antibody production, but immortal) with a specific antibody-producing B cell (antibody production, but mortal). The resulting immortal hybridoma cells are grown in tissue culture and produce antibodies of a single specificity (i.e. monoclonal).

mAb (monoclonal antibody):
antibodies produced by a single clone of B cells, which are hence all identical. They are generated either by immortalizing the antibody-producing B cell or by cloning the respective genes into an expression system.

MAC (membrane-attack complex):
a protein complex composed of the terminal complement proteins, which generates lytic pores in certain pathogens.

MSCRAMMs (microbial surface components recognizing adhesive matrix molecules):
cell wall-attached adhesin proteins, which share a similar protein structure and a common mechanism of ligand binding, including ClfA, ClfB, SdrC, SdrD, SdrE, bone sialoprotein-binding protein, FnBPA, FnBPB and Cna. They mediate the initial attachment of bacteria to abiotic/biotic surfaces, providing a critical step in the establishment and persistence of infections.

Murine antibody:
antibody that has been generated in mice. Murine antibodies are recognized by the human immune system as foreign antigens, and can thus - upon repeated application - lead to allergic reactions, reduced therapeutic effectiveness and shorter circulating antibody half-life.

OPK (opsonophagocytic killing):
the deposition of antibody and/or complement onto the surface of a pathogen makes it more easily ingested by phagocytes.

Passive immunization:
transfer of antibodies / immune sera / immune cells to provide immediate and specific – albeit short-lived – immunological protection.

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Highlights

- *S. aureus* and other staphylococci are the most common cause of persistent biofilm-associated infections, which are inherently resistant to antibiotics as well as the host's immune system.
- Antibody-based approaches can be used to combat biofilms. Antibodies can prevent bacterial attachment and/or biofilm maturation, or even disperse mature biofilms as shown *in vitro* and in pre-clinical studies.
- Several sophisticated techniques can be used for the generation of human monoclonal antibodies, to be ultimately employed in research or clinical settings.
- Since antibodies against surface structures proved unsuccessful in clinical trials so far, current research is focussed on *S. aureus* toxins, and biofilm matrix components.
- Multivalent vaccines, with a special emphasis on biofilm-related targets, are the strategy of choice for active as well as passive immunization.

Outstanding questions box:

- What is the proteome (surface proteins, secreted factors) of staphylococcal biofilms in *ex vivo* or *in vivo*-like conditions?
- How does the adaptive immune system (antibodies, T cells) respond to biofilm as compared to non-biofilm infections?
- How do biofilm-embedded bacteria modulate and subvert innate and adaptive defense mechanisms?
- What are correlates of protection in biofilm infections – type1/3, type 2 or regulatory responses?
- Are specific epitopes of antigens more effective in destabilising biofilms and/or preventing biofilm formation?
- Can the efficacy of monoclonal antibodies be enhanced by using a multivalent vaccine or by combing antibodies with antibiotics or specific enzymes (such as nucleases, proteases)?
- How well can ‘reverse vaccinology’, a genome-based unbiased discovery process for the prediction of candidate vaccine antigens, supplement traditional vaccine approaches?

Box 1: Antibody-based therapies against *S. epidermidis* biofilm-associated infections

S. epidermidis is the most frequent cause of device-related infections, with biofilm formation as the major virulence factor [2,9,121]. Comparable to *S. aureus,* targeting *S. epidermidis* biofilm-associated infections can be achieved either by preventing bacterial attachment to implants, or by blocking cell-to-cell adhesion during biofilm maturation. However, unlike *S. aureus*, *S. epidermidis* biofilm formation relies mainly on exopolysaccharides rather than proteins [20,122].

The two major biofilm matrix constituents, PNAG and Aap, have been targeted by monoclonal antibodies in order to prevent biofilm formation. Anti-PNAG antibodies inhibited biofilm formation *in vitro* and were protective in a rabbit endocarditis model [123]. However, the inhibitory effect on static biofilm formation seems to be strain-dependent [124]. Apparently, PNAG as a biofilm matrix constituent hinders antibody binding close to the bacterial cell surface, which is needed for efficient opsonic killing [45]. The surface-protein Aap promotes cell-to-cell adhesion within a biofilm. Monoclonal antibodies against Aap reduced *S. epidermidis* biofilm formation *in vitro*, but neither enhanced opsonophagocytosis nor protected mice in an experimental biomaterial-associated infection [110,125]. The lack of protection might result from shedded Aap, acting as a decoy for anti-Aap antibodies [125].

The anti-LTA monoclonal antibody Pagibaximab was designed primarily for the treatment of *S. epidermidis* biofilm-associated sepsis, which occurs particularly often in neonates [126,127]. After encouraging results in animals and a more limited phase II study in very low birth weight neonates, a larger phase III study in a similar patient cohort failed to show a reduction in staphylococcal sepsis (Table 2) [108].

As there are no toxins in *S. epidermidis* other than PSMs [128], anti-toxin monoclonal antibody development for the treatment of *S. epidermidis* catheter-related bacteremia has been limited to those peptides. This approach is however problematic due to the diversity and high production of PSMs. Although anti-PSMβ polyclonal antibodies showed some success in limiting the dissemination of *S. epidermidis* biofilm-associated infection in mice [33], an octavalent antigen mixture containing four α-type PSMs, despite their immunogenicity, did not protect against *S. aureus* bacteremia [129], dampening the enthusiasm for passive PSM-targeted vaccination approaches.

A more suitable candidate might be the surface protein SesC, which is expressed in biofilm-associated as well as planktonic cells. Polyclonal rabbit sera against SesC partially prevented *in vitro* biofilm formation by *S. epidermidis* and dissolved established biofilms [130]. A similar reduction in biofilm formation was observed with polyclonal anti-SesC antibodies in a mouse model of catheter-related infections [131].

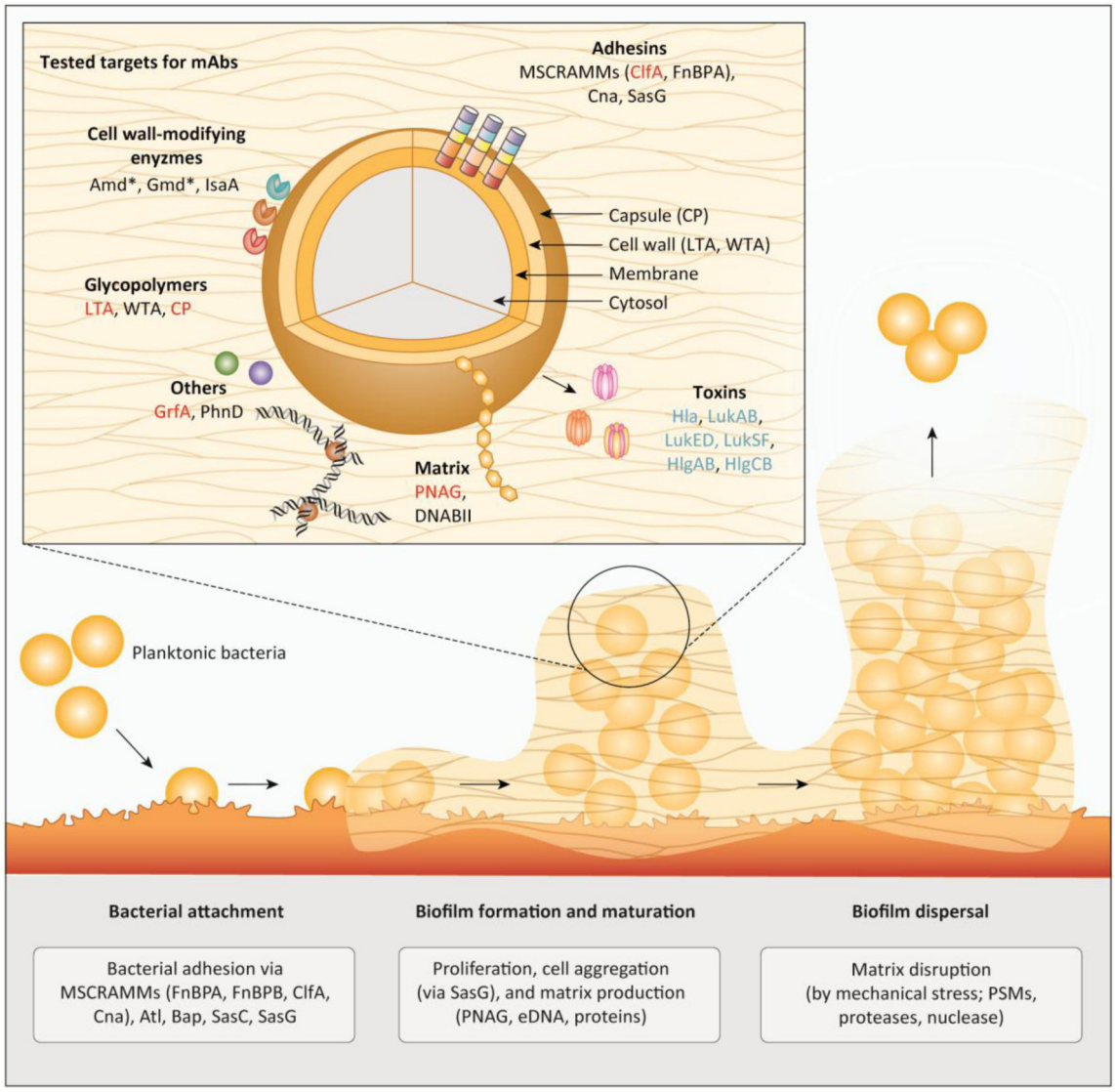

Figure 1: Overview on tested targets for an antibody-based preventive or therapeutic strategy against biofilm-associated *S. aureus* infections.

Main figure: Biofilm formation in staphylococci comprising three main stages: bacterial attachment to a surface, biofilm formation and maturation, and biofilm detachment / dispersal. For the attachment to (a)biotic surfaces, *S. aureus* relies on a broad spectrum of functionally redundant adhesins such as the MSCRAMMs (ClfA, Cna, FnbA, FnbB). After successful adhesion, bacteria start proliferation and production of the biofilm matrix consisting of eDNA, stabilized by DNABII, PNAG and proteins. Eventually, biofilm dispersal is mediated by mechanical shear stress (e.g. in a blood vessel) or by dispersion factors like PSMs, nuclease, and proteases. **Insert:** Molecular targets for antibody based therapies tested in preclinical in clinical studies include adhesins and cell-wall modifying enzymes and other cell wall-attached proteins, surface glycopolymers, biofilm matrix components, as well as toxins and immune evasion proteins. Targets from preclinical studies, ongoing clinical trials and failed clinical trials are shown in black, blue and red, respectively. The asterisk indicates that the *S. aureus* protein autolysin (Atl) is

proteolytically processed into two enzymes, autolysin amidase (Amd) and autolysin glucosaminidase (Gmd), which stay non-covalently attached to the cell surface.

Abbreviations: Atl (autolysin); Amd (autolysin amidase); Bap (biofilm-associated protein); ClfA (clumping factor A); Cna (collagen-binding protein); CP (capsular polysaccharides); DNABII (DNABII family proteins); eDNA (extracellular DNA); FnBPA/FnBPB (fibronectin-binding protein A and B); Gmd (autolysin glucosaminidase); GrfA (ABC transporter); Hla (α-toxin); Hlg (γ-haemolysin); IsaA (Immunodominant staphylococcal antigen A); LTA (lipoteichoic acid); Luk (Leukotoxins); mAb (monoclonal antibody); MSCRAMM (microbial surface components recognizing adhesive matrix molecule); PhnD (subunit of alkylphosphonate ABC transporter); PNAG (poly-*N*-acetyl-ß-(1,6)-glucosamine); PSMs (phenol soluble modulins); Sasc/G (*S. aureus* surface protein C and G); WTA (wall teichoic acid).

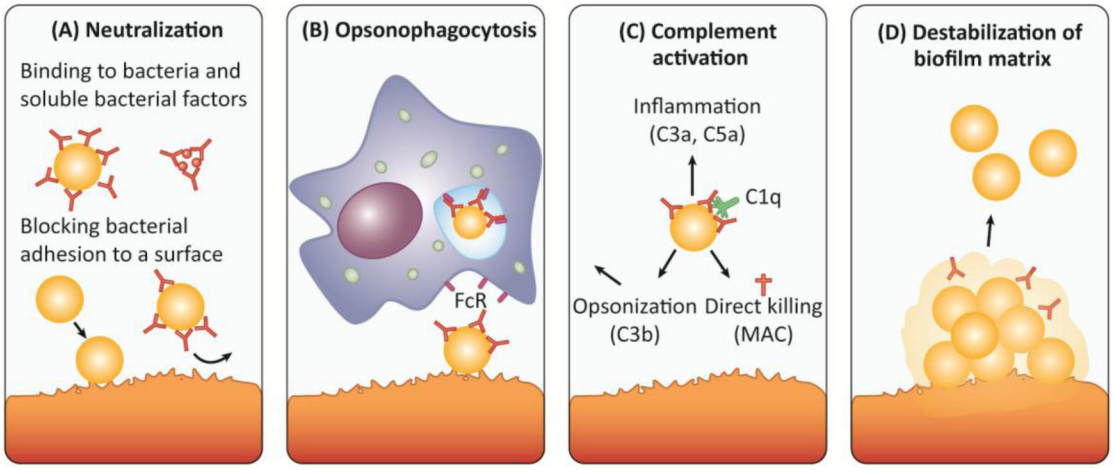

Figure 2: Antibodies can interfere with biofilm formation and promote dispersal of established biofilms by several mechanisms.

A) Secreted staphlyococcoal proteins (e.g. immune evasion molecules, toxins, exoenzymes) as well as surface proteins are involved in biofilm development and are hence potential targets for therapeutic purposes. High affinity IgA and IgG antibodies can neutralize the action of bacterial toxins and surface proteins. Moreover, antibodies can bind to bacterial adhesins (e.g. ClfA, FnBPA) and cell wall components (e.g. PNAG), thereby blocking initial attachment to host matrices and subsequent initiation of biofilm formation. **B)** Surface-bound antibodies (most prominently IgG) can trigger the uptake and destruction by neutrophils and macrophages expressing Fc receptors (FcR) on their surface (opsonophagocytosis). Activation of neutrophils can also trigger granule release, oxidative burst and NETosis. **C)** Surface-bound antibodies (IgM and IgG) trigger complement activation via the classical pathway. Following binding of C1q to the surface-bound antibody, the complement cascade is initiated resulting in the formation of the C3 convertase, which cleaves the central component of all complement pathways, C3, into C3a and C3b. C3b acts as an opsonin, enabling phagocytes that express the C3b receptor to ingest C3b-coated bacteria more easily. The soluble C3a (as well as C5a) act as chemo-attractants that recruit immune cells to the site of infection causing inflammation. C3 activation also triggers the formation of the membrane attack complex (MAC) that generates lytic pores in certain pathogens. Gram-positive bacteria, including *S. aureus,* are protected from MAC-dependent lysis by their thick peptidoglycan layer [132]. **D)** Antibodies targeting different components of the biofilm matrix, e.g. DNABII, can destabilize a biofilm matrix and thereby promote bacterial dispersal and clearance by immune cells or antibiotics. Abbreviations: FcR (Fc receptor); MAC (membrane attack complex).

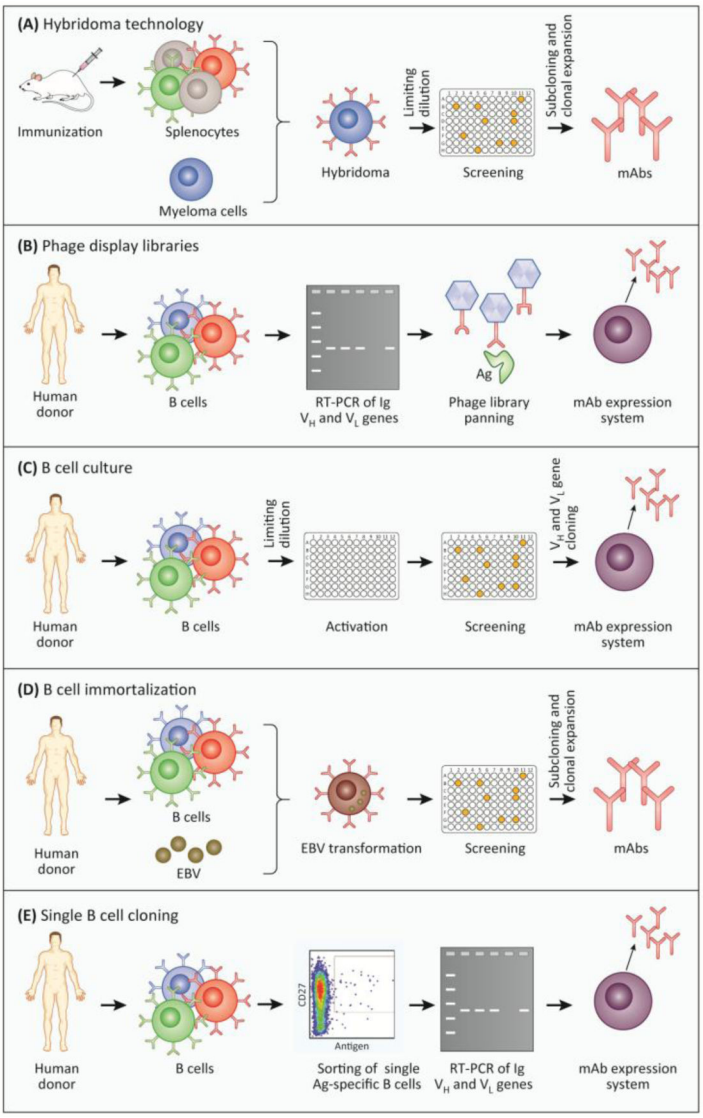

Figure 3: Methods commonly used for the generation of monoclonal antibodies (mAbs).

A) Hybridoma technology. Following immunization with an antigen, mice start producing large amounts of antigen-specific B cells. These cells are harvested from the spleen and fused with myeloma cells. The resulting hybridoma cells are screened for the secretion of antigen-specific antibodies. Antigen-specific hybridoma cells are selected by limiting dilution (subcloning) [3]. **B) Phage display library.** Initially, mRNA is isolated from B cells or plasma cells and then reverse-transcribed into cDNA. The variable light and heavy chains are amplified via PCR and ligated into a phage display vector. The resulting phage library consists of 10^8-10^{10} different phages, each encoding a single surface-expressed mAb, generated by random combination of heavy and light chains. The antigen is subsequently "displayed" to the phage library in successive rounds, to enrich antigen-specific phages (panning). The genes encoding the desired antigen-specific mAbs can then be cloned into an appropriate expression system for the generation of the mAbs of interest [1,2]. **C) B cell culture.** After isolation and limiting dilution, B cells are cultivated and activated *in vitro*

leading to the secretion of antibodies. B cell culture supernatants are screened for antigen-specific antibodies, and positive cultures are used for the amplification of heavy and light Ig genes via PCR. The antibody sequence is finally cloned into an expression system to produce the mAbs [6]. **D) EBV immortalization.** Human B cells or plasma cells are isolated and immortalized using Epstein-Barr virus followed by subsequent single cell distribution. Supernatants of B cell cultures are screened for specific antigen binding and subsequently subcloned to produce mAbs [4]. **E) Single B cell cloning.** Human B cells are isolated and single antigen-specific B cells are sorted by fluorescence-activated cell sorting (FACS). The mRNA of those single cells is reverse-transcribed into cDNA followed by amplification of Ig heavy and light chains via PCR. The extracted antibody sequences can be cloned into a vector and ultimately introduced into an expression system. Finally, the resulting monoclonal antibodies are validated for their antigen-specificity [5].
Abbreviations: Ag (antigen); EBV (Epstein-Barr virus); Ig (Immunoglobulin); mAb (monoclonal antibody).

Table 1:

Molecular targets for antibody-based therapies against *S. aureus* biofilms in pre-clinical studies.

Target[a]	*In vitro* anti-biofilm activities[b]	Protection in *ex vivo* / *in vivo* biofilm models[c]	Antibody[d]	Reference(s)
Adhesins, including MSCRAMMs				
ClfA	block FBG binding / agglutination of human plasma; displace FBG-bound bacteria; promote OPK	**P:** full - rabbit IE model **T:** partial (+vancomycin) - rabbit IE model	Mu/mAb (MAb 12-9. 11H10), Huz/mAb (tefibazumab)	[69,70]
FnBPA	block FBN binding; promote OPK and nGr activation; reduce biofilm formation	Not tested	Mu/mAb	[63,64]
Cna	block CN binding; displace CN from bacterial surface; promote OPK; block laminin and C1q binding	**P:** strong - sepsis model (less animals developed arthritis)	Murine pAb, Mu/mAb	[133–135]
SasG	reduce biofilm formation	Not tested	Rabbit pAb	[136]
Cell wall-modifying enzymes				
Atl[1]	inhibit biofilm formation; promote OPK	Not tested	Murine pAb	[71,72,137] https://www.ncbi.nlm.nih.gov/pubmed/27044299
Atl-Amd	promote OPK	Not tested	Murine pAb	[72]
Atl-Gmd	promote OPK; block cell division (binary fission); induce agglutination	**P:** strong - murine model of implant-associated osteomyelitis	IgG1 Mu/mAb (1C11)	[73]
IsaA	promote nGr activation (ox. burst) and OPK by nGrs (UK-66); promote OPK In whole blood (hUK-66); promote nGr activation, but not phagocytosis (1D9)	**P:** partial, strain-dependent (1D9) - murine bacteremia model **T:** no (1D9) - murine bacteremia model; partial (UK-66) - catheter-related infection model	Mu/mAb (UK-66), Huz/mAb (hUK-66) Hu/mAb (1D9)	[138–140]
Glycopolymers				
WTA	promote C3 deposition and OPK by nGr (hu pAb)	**P:** no - murine bacteremia model (Hu/mAb)	Hu/mAb, IgG Hu/mAb (THIOMAB)	[61,75]
CP	promote OPK (mu/mAb)	**P:** partial – rat endocarditis model (Ra/pAb); no protection – rat endocarditis model (Mu/pAb)	Mu/mAb, Ra/pAb, Mu/pAb	[77–79]
LTA	promote OPK	Not published	murine/human chimeric mAb (Pagibaximab®)	[141]
Matrix components				

Target[a]	*In vitro* anti-biofilm activities[b]	Protection in *ex vivo* / *in vivo* biofilm models[c]	Antibody[d]	Reference(s)
PNAG / dPNAG	promote OPK	**P:** partial - murine bacteremia model	IgG1 Hu/mAb (F598)	[29,65]
DNABII	disrupt established biofilms	**T:** partial (+daptomycin) - murine tissue cage infection model **T:** partial (+vancomycin) - rat IE model	native Hu/mAb (TRL1068)	[84,85]
Immune evasion proteins				
Spa	neutralize Fcγ and V_H3+ Fab binding activities of Spa; promote OPK in mouse and human blood	**P:** strong - murine sepsis model with renal abscess formation	Mu/mAb against Spa toxoid (3F6)	[142]
Toxins				
Hla	neutralize toxin activity; modestly inhibit biofilm formation	**P:** complete - *ex vivo* porcine vaginal mucosa explants **P:** partial - hematogenous orthopedic implant infection in mice (increased protection in combination with anti-ClfA 11H10 mAb)	Hu/mAb (MEDI4893)	[62,91,92]
LukAB	neutralize LukAB-mediated cytotoxicity; inhibit LukAB binding to I domain of CD11b	**P:** partial - murine sepsis model (1:1 mixture of SA-15 and SA-17)	Hu/mAb (SA-13, -15 and -17)	[96]
Other proteins				
PhnD	inhibit biofilm formation under shear flow (*S. aureus* and *S. epidermidis*), promote OPK by nGr	Not tested	Ra/pAb	[143]

[a] Protein names: ClfA (clumping factor A); FnBP (fibronectin-binding protein); Cna (collagen-binding protein); SasG (*S. aureus* surface protein G); Atl (autolysin: bifunctional enzyme that undergoes proteolytic cleavage to yield two catalytically active proteins, an amidase (Amd) and a glucosaminidase (Gmd), which both are involved in peptidoglycan cleavage); IsaA (Immunodominant staphylococcal antigen A); WTA (wall teichoic acid); CP (capsular polysaccharides); LTA (lipo teichoic acid); PNAG/ dPNAG, PNAG (poly-*N*-acetyl-ß-(1,6)-glucosamine); dPNAG (deacetylated PNAG); DNABII (DNABII family proteins); Spa (staphylococcal protein A); Hla (α-toxin); LukAB (Leukotoxins A and B); PhnD (subunit of alkylphosphonate ABC transporter).

Footnotes:

[b] FBG: fibrinogen; FBN: fibronectin; CN: collagen

[c] P (prophylaxis); T (therapy); IE (infective endocarditis)

[d] Abbreviations: mAb (monoclonal antibody); Hu/mAb (human mAb); Huz/mAb (humanized mAb); Mu/mAb (murine mAb); Ra/mAb (rabbit mAb); pAb (polyclonal antibodies); Hu/pAb (human pAb); Mu/pAb (murine pAb); Ra/pAb (rabbit pAb); IHF (integration host factor)

Table 2:

Clinical trials involving therapeutic antibodies/antisera against *S. aureus* infections [*]

Target[a]	Name [Company; NCT number[b]]	Study Design	Status (study result)	Intervention[c]	Ref
Single component					
ClfA (adhesin)	Tefibazumab (Aurexis®) [Inhibitex]	randomized, double-blind, placebo-controlled trial of bacteremia patients receiving standard antibiotic treatment plus Tefibazumab (N = 63)	Phase II (failed)	Huz/mAb (IgG1)	[104]
	Tefibazumab (Aurexis®) [Inhibitex; NCT00198289]	dose escalation study of Aurexis® in cystic fibrosis patients chronically colonized with *S. aureus* in their lung (N = 30)	Phase IIa (failed)	Huz/mAb (IgG1)	*i*
CP 5 and CP8 (capsular polysaccharides)	AltaStaph™ [Nabi Biopharmaceuticals; NCT00063089]	randomized, double-blind, placebo-controlled trial involving adult *S. aureus* bacteremia patients receiving standard treatment plus Altastaph (N = 40)	Phase II (halted)	polyclonal human IgG with high antibody titers against CP5 and CP8, purified from the plasma of healthy donors that have been vaccinated with StaphVAX[d]	[105]
	AltaStaph™ [Nabi Biopharmaceuticals; NCT00066989]	randomized, double-blind, placebo-controlled trial for prevention of nosocomial *S. aureus* infections in very low birth weight (VLBW) neonates (N = 206)	Phase II (failed)	same as above	[106]
LTA (cell wall component)	Pagibaximab® [Biosynexus; NCT00631800]	randomized, double-blind, placebo-controlled dose-ranging study on prevention of CoNS and *S. aureus* sepsis in VLBW neonates (*N* = 88)	Phase II (finished)	murine/human chimeric mAb	[127]
	Pagibaximab® [Biosynexus; NCT00646399]	randomized, double-blind, placebo-controlled study on prevention of staphylococcal sepsis in VLBW neonates (N = 1579)	Phase III (failed)	murine/human chimeric mAb	[108]
WTA (wall teichoic acid)	DSTA4637S [Roche/Genentech; NCT03162250]	randomized double-blind, placebo-controlled multiple-ascending dose study on safety, tolerability, and pharmacokinetics in *S. aureus* bacteremia (N = 24)	Phase lb (ongoing)	THIOMAB™ antibody (Hu/mAb; IgG1)-antibiotic conjugate	[114,115]
PNAG (cell wall component)	SAR279356 [Sanofi-Aventis; NCT01389700]	randomized, double-blind, placebo-controlled study to assess a single dose of SAR279356 in ICU patients on mechanical ventilation (N = 7)	Phase IIa (terminated due to difficulty in patient recruitment)	Hu/mAb	*ii*
Hla (toxin)	MEDI4893 (Suvratoxumab) [MedImmune LLC; NCT02296320]	randomised, double-blind, placebo-controlled, single-dose, dose-ranging study in mechanically ventilated adult subjects (N = 213)	Phase II (ongoing)	Hu/mAb (IgG1)	[111]

Target[a]	Name [Company; NCT number[b]]	Study Design	Status (study result)	Intervention[c]	Ref
	AR-301 (Salvecin®) [Aridis Pharmaceuticals; NCT01589185]	randomized, double-blind, placebo-controlled, single dose study of AR-301 as an adjunctive therapy against severe *S. aureus*-related pneumonia (N = 48)	Phase IIa (successful)	Hu/mAb (IgG1)	*iii*
GrfA (ABC transporter)	Aurograb® [NeuTec Pharma Ltd./Novartis Pharma AG; NCT00217841]	randomised, double-blind, placebo-controlled trial of patients with severe, deep-seated staphylococcal infections receiving vancomycin plus Aurograb (N = 180)	Phase II (failed), development stopped	single chain antibody fragment (Fab)	[134,144]
Multi-component					
ClfA (*S. aureus*), SdrG (*S. epidermidis*) (adhesins)	INH-A21 (Veronate®) [Inhibitex / Bristol Myers-Squibb; NCT00113191]	randomized, double-blind, placebo-controlled study of INH-A21 for prevention of staphylococcal late-onset sepsis in VLBW infants (N = 1983)	Phase III (failed)	Pooled human Ig purified from the serum of donors with high titers against ClfA and SdrG	[113]
Hla, HlgAB, HlgCB, LukED, LukSF, LukAB (toxins)	ASN100 [Arsanis Biosciences GmbH; NCT02940626]	randomized, double-blind, single-dose, placebo-controlled study of ASN100 for the prevention of *S. aureus* pneumonia in heavily-colonized, mechanically ventilated subjects (N = 354)	Phase II (halted)	Hu/mAb combination of ASN-1 (IgG1, crossreactive mAb with affinity for Hla, HlgAB, HlgCB, LukED and LukSF) and ASN-2 (IgG1, mAb against LukAB)	[95,112]

*data based on publications, review of sponsor website information as well as clinical trial data accessible via www.clinicaltrials.gov on 20.08.2018.

[a]**Abbreviations:** ClfA, clumping factor A; LTA, lipoteichoic acid; PNAG, ß-1,6-poly-*N*-acetylglucosamine; Hla, α-toxin; SdrG, Serine- aspartate repeat- containing protein G; HlgAB and HlgCB, γ-haemolysin AB and CB; LukED and LukSF, leukotoxin ED and SF; NA, data not available; VLBW, very low birth weight.

[b]ClinicalTrials.gov Identifier[iv]

[c]Antibodies applied in clinical studies were either murine, chimeric, humanized or human (see Glossary)

[d]StaphVAX® is a bivalent *S. aureus* vaccine which contains the purified capsular polysaccharides (CPS) types 5 and 8[v]. Its development was halted by Nabi due to failure in preventing *S. aureus* infections in kidney disease patients in a confirmatory phase III clinical trial[vi].. The company also halted the development of Altastaph™, as it is based on the same capsular polysaccharide technology as StaphVAX®.

Resources

[i]https://clinicaltrials.gov/ct2/show/NCT00198289

[ii]https://www.sanofi.com/media/Project/One-Sanofi-Web/sanofi-com/common/docs/clinical-study-results/PKD11791_summary.pdf

[iii]https://aridispharma.com/ar-301/

[iv]https://clinicaltrials.gov/

[v]https://clinicaltrials.gov/ct2/show/NCT00211913?term=StaphVAX&rank=1

[vi] https://www.sec.gov/Archives/edgar/data/72444/000119312505214434/dex99.htm