B13: "Are monoclonal antibodies capable of eradicating biofilms in orthopedic infections?"

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RESPONSE/RECOMMENDATION: While there is preclinical evidence that monoclonal antibodies (mAbs) can disrupt biofilm structure, passive immunization clinical trials for orthopaedic infections have only reached phase 1. Thus, further preclinical as well as clinical research is needed to explore the full potential and the efficacy of mAb therapy.

LEVEL OF EVIDENCE: moderate

DELEGATE VOTE: Agree: [% vote], Disagree: [%], Abstain: [%]

RATIONALE: Periprosthetic joint infections (PJI) pose varied and significant challenges in clinical settings due to their persistent nature, the complexity and prolonged duration of treatment[1]. Among the formidable barriers to effective management of these infections are biofilms—structured communities of bacteria that adhere to both living and non-living surfaces, including orthopedic implants such as screws, plates, and prosthetic joints, significantly complicating infection management[2; 3]. Biofilms are notably resistant to standard antibiotic therapies and host immune responses, leading to chronic infections and prolonged recovery times, increased morbidity, and higher healthcare costs[4; 5]. This resilience of biofilms necessitates the exploration of novel therapeutic strategies capable of penetrating these barriers and effectively eradicating the bacteria within[6]. Monoclonal antibodies (mAbs) against bacteria and biofilm targets have been investigated for this purpose, as they are highly specific for a single antigen, and have revolutionized treatment in various diseases, including oncology, rheumatology, and infectious diseases[7].

To answer the question of whether monoclonal antibodies are capable of eradicating biofilms in orthopedic infections, a comprehensive literature search was conducted using the search words "monoclonal antibody" and "biofilm" within PubMed and Embase, which identified 190 unique studies, 27 of which were formally reviewed to answer this question.

In infection research, mAbs can be designed to target specific pathogens or pathological processes, rendering them suitable candidates for addressing the unique challenges posed by biofilms[8]. Since they function through mechanisms such as the neutralization of target molecules, the recruitment of immune effector cells, and through direct antimicrobial effects, they can be effectively used to disrupt the integrity of the biofilm matrix, promote opsonization, enhance immune clearance by exposing bacterial cells to immune cells, and inhibit the signals that promote biofilm growth and maintenance[3; 9-16]. Consequently, they offer a potential targeted approach to overcome the limitations of traditional anti-infective treatments.

Indeed, preliminary studies have shown that combinational treatment strategies involving mAb together with conventional antimicrobial therapies show enhanced effectiveness and reduced bacterial persistence[17]. This stems from the fact that mAbs can disrupt biofilm formation or trigger biofilm dispersal, thereby allowing the co-administered antibiotic to more effectively kill the bacteria released from the biofilm, ultimately providing a more successful clinical outcome, while promoting antimicrobial stewardship[18].

The mAb that have demonstrated potential in targeting different components of biofilms include:

- Anti-DNABII mAb: The DNABII family of proteins includes integration host factor (IHF) and histone-like (HU) proteins. These proteins bind extracellular DNA (eDNA) released by bacteria and mammalian cells, forming a crosslinked scaffolding that stabilizes the biofilm extracellular matrix. Targeted mAb against DNABII disrupts this DNABII-eDNA scaffolding, resulting in the collapse and dispersal of the biofilm matrix—this has been demonstrated in in vitro and in vivo preclinical studies,

including implant-associated infections [9; 23-26]. Disruption of DNABII proteins using monoclonal antibodies (mAbs) promotes the transition of bacteria from a dormant biofilm-associated state to a more susceptible planktonic form, thereby enhancing antibiotic efficacy[9]. TRL1068 is a fully human anti-DNABII mAb. Most recently, a phase 1 clinical trial of TRL1068 therapy in patients with PJI was completed and demonstrated the safety and feasibility of this passive immunization approach [27]. CMTX-101 is an anti-DNABII humanized mAb derived from a murine mAb, which has recently completed a phase 1a clinical trial showing safety in healthy volunteers, a phase 1b study in moderate-severe community-acquired pneumonia patients. A phase 1b/2a study in patients with cystic fibrosis chronically infected with *Pseudomonas aeruginosa* is currently being conducted with CMTX-101.

- Anti-PNAG antibodies: These target poly-*N*-acetylglucosamine (PNAG), a substance that mediates cell-to-cell adhesion within biofilms. mAbs against PNAG have been shown to reduce biofilm formation and enhance bacterial clearance, offering a promising target for immune-based therapies[22].
- mAb 3H3: This antibody targets amyloids, specifically curli fibers, which are major components of biofilms in Enterobacteriaceae. By disrupting the amyloid structure within biofilms, mAb 3H3 enhances the efficacy of antibiotics and facilitates more efficient clearance of infections[23].
- Anti-Atl mAb: Functionally, *S. aureus* autolysin (Atl) has been shown to be essential for cell wall biosynthesis and degradation during binary fission[24-26], and several research groups have identified Atl as an immunodominant antigen[27; 28]. Atl has also been shown to function as an adhesin[29], and a biofilm enzyme[30]. Preclinical studies have demonstrated the efficacy of mAbs against the glucosaminidase (Gmd) subunit of Atl in murine models of implant-associated osteomyelitis[31; 32], and the feasibility of anti-Gmd passive immunization in sheep[33].
- MEDI4893*, 11H10: α-toxin (AT) and clumping factor A (ClfA) are virulence factors of *S. aureus* which were identified as key pathogenic factors of *S. aureus* infections by promoting biofilm formation. Hence, neutralizing human mAbs against these factors (MEDI4893* and 11H10, respectively), when used in combination, inhibited biofilm formation *in vitro* and in a murine model of *S. aureus* hematogenous implant-related infection, thus providing a targeted, non-antibiotic alternative approach to help prevent these types of infections[11].
- Multi-mechanistic mAb combination (AZD6389*): a combination of three mAbs targeting specific staphylococcal virulence factors, namely AT, bicomponent cytotoxins (LukSF/LukED/HlgAB/HlgCB), and ClfA was shown to have protective efficacy when tested in a rabbit model of methicillin-resistant *S. aureus* PJI, showing significant reductions in bacterial burden and inflammatory signs (joint swelling, erythema, intra-articular pus)[9]. Such an approach might therefore have potential clinical utility as a pathogen-targeted approach for the prevention of *S. aureus* PJI.
- Conjugated mAbs: Another interesting application of mAbs is their use as vehicles to facilitate targeted delivery of therapeutics (e.g. radiopharmaceuticals, antibiotics, …) to the site of infection, in what is known as antibody-based biologicals. Such an approach would ideally allow clinicians to precisely target pathogens, without damaging surrounding healthy tissues, thus providing an alternative approach to improve the diagnosis and/ or treatment of biofilm-related infections and enhancing overall treatment efficacy.

An innovative option is radioimmunotherapy (RIT), which efficiently combines the precision and specificity of mAbs with the cytotoxic power of radionuclides. Therefore, by using mAbs which specifically recognize antigens expressed uniquely or significantly overexpressed on the surface of pathogens, one would be able to specifically direct the cytotoxic effects of radionuclides to those organisms and their biofilms with high precision, thereby achieving a local bactericidal effect. RIT could therefore be used as an alternative treatment modality against biofilm-related microbial infections, with and without conventional therapies such as antibiotics.

One prominent example of these target structures is wall teichoic acids (WTA), which are anionic polymers covalently bound to the peptidoglycan layer of many Gram-positive bacteria, and in the extracellular matrix of biofilms. Ye et al successfully linked mAbs (anti-β-GlcNAc antibody 4497-IgG1), designed for targeting WTA found on *S. aureus* and its biofilm, to radionuclides, and demonstrated their

potent anti-bacterial effects against both planktonic and biofilm-associated *S. aureus in vitro*[15]. Similarly, van Dijk et al used radioimmunotherapy using the same human mAb (anti-β-GlcNAc 4497-IgG1) for non-invasive localization of the infected implant in a murine subcutaneous implant infection model, as well as for treatment of *S. aureus* implant-associated infections, where they observed a dose dependent killing of MRSA in both planktonic and biofilm state[12; 13]. However, further *in vivo* studies are necessary to validate the *in vitro* observed bactericidal effect of RIT on *S. aureus*, and to evaluate the safety and efficacy of this approach in clinical settings involving implant associated infections.

Another novel and very promising approach to prevent antibiotic treatment failure involves conjugating a mAb to an antibiotic, producing an antibody-antibiotic conjugate (AAC), which can be used for targeted delivery of potent antibiotics into host cells to achieve bactericidal concentrations intracellularly, and hence holds promise for the therapy of orthopedic implant-associated intracellular *S. aureus* infections. For instance, Qin et al conjugated a human mAb (M0662) which directly recognizes staphylococcal protein A (a highly conserved surface protein), with the antibiotic vancomycin, which lacks the ability to penetrate host cells[10]. This AAC significantly improved the bactericidal effects of vancomycin, both *in vitro*, and in a murine implant infection model, where it accelerated the clearance of *S. aureus*, prevented pathological changes in trabecular bone structure, and effectively blocked the formation of bacterial biofilm on the implant.

Conclusion: Monoclonal antibodies represent a revolutionary approach to combating the resilience of biofilms in infectious diseases. By targeting specific structural components of biofilms, these antibodies not only disrupt the biofilm but also can restore antibiotic sensitivity to otherwise resistant bacteria by removing the physical barrier of the biofilm and by increasing the metabolic activity of the resident bacteria.. The ongoing research and development in this field hold significant promise for the management of chronic orthopedic infections.

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