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Monoclonal Antibody-Based Therapies for Bacterial Infections

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Abstract

Purpose of review—This review highlights recent developments in the development of monoclonal antibodies to treat bacterial disease, including preclinical advances and the status of current clinical trials.

Recent Findings—Monoclonal antibody (mAb) therapy is becoming increasingly promising in the infectious disease field. Though bacterial exotoxins continue to be a mainstay of mAb targets, searches for protein targets on the surface of bacteria have uncovered new mechanisms of antibody-mediated action against bacteria. Additionally, surveys of the polysaccharide serotype prevalence among antibiotic resistant bacterial populations have yielded opportunities to leverage human selective pressures to our clinical advantage. Several mAb candidates are progressing through clinical development with great promise, especially those with structures altered to provide maximum benefit. While other clinical trials have recently proved unsuccessful, these failures and lessons from immune profiling provide opportunities to understand how vulnerabilities of certain targets may change in different disease states.

Summary—Despite the hurdles of identifying effective targets and understanding how mAbs provide protection within different infections, we show that the progress made in these fields is a positive indication of mAbs becoming more widely accepted as the future for treating bacterial infections.

Keywords

Monoclonal Antibody Therapy; Antibiotic Alternatives; Infections

INTRODUCTION

From their initial development by murine hybridoma technology, to advancements in screening and modern engineering of humanized antibodies, monoclonal antibodies (mAbs) have grown rapidly in their therapeutic potential (1). Over 70 mAbs have been approved for

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human use, and eight times that many are in clinical development (2). Furthermore, with traditional antibiotics becoming increasingly obsolete due to antimicrobial resistance (AMR), mAbs are making a comeback in the field of anti-infective drugs alongside phage therapy and other historic strategies (1, 3). After being overshadowed for years by successes in anti-cancer and anti-immune antibody therapies, efforts to engineer mAbs against pathogens have finally yielded fruit, with four FDA certifications and a growing number of promising clinical trials (3, 4). However many hurdles remain in the field of anti-infective mAbs: finding optimal targets for a pathogen, understanding how the Fc receptor (FcR), isotype, and other structural regions mediate protection, and developing better pre-clinical and clinical trials to investigate the therapeutic potential of these antibodies. This review examines recent efforts pertaining to these pursuits.

Antibodies against Bacterial Toxins

Antibody therapies against infections have targeted numerous bacterial epitopes and virulence factors (Figure 1), the first efforts focusing primarily on toxin neutralization. Indeed, all three currently-licensed FDA therapies against bacteria target bacterial exotoxins (4). Anti-toxin mAb therapies are thought to inhibit the virulence of the organism to limit invasion or damage to the host, without creating selective pressures on the organism.

In past years attempts to generate mAbs against toxins of *Staphylococcus aureus* (5–9), *Streptococcus pyogenes* (10), *Clostridia* species (11, 12), and *Escherichia coli* (13) have been undertaken, with variable success. The FDA-approved bezlotoxumab [Zinplava, Merck, Kenilworth NJ] which targets *C. difficile* TcdB, is currently approved to prevent the recurrence of *C. difficile* infection, but has not been shown to cure active infection (11). More recently, clinical studies of mAb MEDI4893 [Medimmune, Gaithersburg MD] demonstrate it to reach levels in the blood and nares capable of neutralizing *S. aureus* alpha-hemolysin to prevent invasion (14). Thanks to the high conservation of the alpha-hemolysin (15), the therapy is likely immune to resistance, but its inability to alter *S. aureus* colonization or bacterial expression may limit it use to prophylaxis (14). Thus, while anti-toxin mAbs seem effective as preventative strategies or as adjunctive treatments to improve antibiotic success (7), their ability to directly treat acute disease may be limited. The limitation could be overcome by coupling anti-toxin immunologics with those with direct activity against the bacteria, such as through cocktails or bispecific antibodies. Care should be taken in the case of bispecific antibodies however, as the proximity of its two targets *in vivo* must be considered (16).

Pursuits of anti-toxin mAb therapy were likely frustrated this summer by announcement that Arsanis [Waltham, MA] will discontinue development of its ASN100 mAb cocktail. Despite the ability of the two component antibodies in neutralizing numerous *S. aureus* cytotoxins and successes in *in vitro* and *ex vivo* models (8)*, the Phase II study testing ASN100s ability to prevent *S. aureus* pneumonia in mechanically ventilated patients was ended prematurely due to its predicted failure to meet its primary endpoint, unsuccessful (Table 1). However, the advancement of AR301 [Aridis, San Jose CA] to Phase III testing this January is an encouraging sign that anti-toxin therapies will continue to yield success.

Antibodies against Surface Proteins

Much interesting work has been performed on antibodies targeting outer membrane proteins (OMP) of bacteria, including proteins involved in adhesion (17–20), immune evasion (21, 22), and bacterial biosynthesis (23, 24). Many of these proteins are integral to the function of these bacteria, which make them not only effective but also easier targets as they are more likely conserved across clinical strains (20). The most successful of these as of today has been MEDI3902 [MedImmune], a bispecific antibody against *Pseudomonas aeruginosa* fimbrial protein PcrV and exopolysaccharide Psl, both of which were found to be conserved across *Pseudomonas* clinical isolates (25). Preclinical work has shown MEDI3902 to successfully treat rabbits with acute *P. aeruginosa* pneumoniae, improving survival and lung oxygenation as well as decreasing organ bacterial burden and pathology (19). Additionally, Phase I tests showed that serum-levels of the antibody after administration were sufficient to promote complement-dependent opsonophagocytic killing (OPK) of *Pseudomonas in vitro* (18). Although this effect may be reduced in the lung due to the reported low tissue distribution, this mAb holds promise of being one of the first in clinical development to utilize its Fc-mediated functions of antibodies to treat disease, and a Phase II study of the drug to prevent infections in mechanically ventilated patients has begun recruiting.

Disabling immune evasion proteins has also been a popular strategy, especially in the context of Protein A of *S. aureus* (SpA) (21, 22). Under normal circumstances, SpA cripples antibody immunity by binding the Fc region to prevent proper orientation while also shifting B cell responses to produce less-protective antibodies. However, the virulence factor's inability to bind the human IgG_3 subtype was exploited to identify an opsonophagocytic antibody 514G3, which was demonstrated to have effective *in vivo* prophylaxis against MRSA bacteremia, as well as synergy with vancomycin to reduce lethality (22). The antibody has already shown in a Phase I-II study to reduce hospitalization times in patients with MRSA bacteremia (26), and is planned to be tested in a Phase II clinical study this year. Similarly, a recombinant antibody developed from a non-toxigenic SpA vaccine reduced MRSA-mediated disease in mice (21). More importantly however, periodic systemic administration of the mAb was shown to progressively reduce nasal and gastrointestinal colonization of MRSA (21), a capability with significant clinical implications. Patients found with infections by AMR pathogens such as *Staphylococcus aureus* (27) or *Klebsiella pneumoniae* often were found to be previously colonized with the same organism (28). As a result, many strategies using antibodies and derived nanobodies have focused on reducing rates of patient and animal colonization (29).

Porins have also been popular targets for strategies against gram negative organisms (30, 31). A rodent IgG targeting *Escherichia coli* BamA was interestingly found to possess complement-independent bactericidal activity, which previously had only been observed in antibodies against *Borrelia* (32, 33)**. The mAb, MAB1, additionally showed insights on how membrane fluidity affects interactions with surface proteins, which future efforts at mAbs against surface targets should consider. However, the initial design of MAB1 demonstrated how surface proteins, despite being well-conserved and efficacious targets, are often concealed by an abundance of variable polysaccharides (31, 34).

Antibodies against Polysaccharides

Polysaccharide targets, including lipopolysaccharide (LPS) and capsular polysaccharide (CPS), have been popular targets since immunotherapy's infancy. CPS, for example, is a necessity for many bacteria seeking to avoid host immunity, making it among the most effective targets in vaccine development. Antibodies that bind CPS improve the opsonophagocytosis of normally 'slippery' bacteria (35, 36), and have even been shown to directly affect bacterial metabolism as well (37). Consequently, the selection pressure these polysaccharides are under is tremendous, and as a result most polysaccharides are extremely variable, presenting a challenge when designing mAbs (38, 39). Whereas covering multiple serotypes is standard in vaccines, doing so with mAbs is more difficult; most mAbs used in human therapy are highly-specific IgG isotypes to meet dose requirements, limiting cross-reactivity and necessitating cocktails of multiple mAbs. Antibodies of IgM and IgA isotypes may provide better protection in these scenarios, as these isotypes are thought to be more cross-reactive due to their lack of affinity maturation. Such rationale has been recently challenged by studies of natural LPS antibodies, which found a high frequency of somatic hypermutations in IgM and IgA against certain glycan signatures (40). Additionally, such isotypes are multimeric, compounding multiple low affinity interactions to ultimately reach a high functional avidity. The caveat is IgMs are large molecules with shorter half-lives and higher side effect risks, which have made them less desirable as lead candidates. Additionally, any successful mAb therapy against a polysaccharide antigen may ultimately shift bacterial populations away from utilizing that antigen, as has been observed across *S. pneumoniae* strains in response to vaccination (37).

Ironically, through the creation of selective pressures favoring AMR isolates, selection pressures favoring diversity of polysaccharides in some species seems to have waned, providing opportunities at broadly-reactive antibodies (39). The comparatively high conservation of CPS within ST238, the most endemic clone of carbapenem-resistant *Klebsiella pneumoniae* (CR-Kp) in the United States, have allowed us and others to find antibodies that react with a subclade of strains that comprises at least half of stains within this clone (35, 36)**. Similarly, conservation within ST258 LPS has allowed for the development of mAbs that protects against endotoxin-mediated lethality in sensitized mice and rabbits infected with CR-*Kp* from either subclade (41)*. Other *Enterobacteraciae* demonstrate conservation of polysaccharides among AMR clones as well. The O25b variant of *Escherichia coli* LPS is conserved within ST131-H30 strains, which comprise up to a quarter of all extraintestinal infections by *E. coli*, and has been successfully targeted by a humanized antibody to limit bacteremic infection by endotoxin neutralization and complement-mediated killing (42). Such studies exemplify how effective anti-polysaccharide mAb therapy can be in the absence of antigenic variability and provide exciting strategies for future clinical development of anti-polysaccharide mAbs against AMR organisms.

Interestingly the problem of carbohydrate variability is conspicuously absent in the context of *Pseudomonas aeruginosa,* as the alginate-based *Pseudomonas* capsule and Psl exopolysaccharide have been remarkably robust targets that are succeeding in clinical studies (18, 19, 25, 43, 44) Studies by Zaidi and colleagues showed the ability of antibodies

against alginate and another conserved polysaccharide, poly-N-acetylglucosamine (PNAG), to reduce pathology and bacterial burden by gram positive cocci or *Pseudomonas* respectively in a novel model of bacterial conjunctivitis through the action of microbiome-matured lymphocytes (43). This data indicates not only viable targets for mAb therapy but suggests that innate immunity may not monopolize mechanisms of mAb-mediated protection. PNAG and other teichoic-acid motifs polysaccharides have been found to be broadly expressed across a variety of different pathogens, and recent work has suggested the potential of mAbs against PNAG as a broad antimicrobial therapy (43, 45), Success in the clinic has been mixed. The mAb F598 [Alopexx, Concord MA] has met numerous hurdles in clinical development, as both Phase II clinical studies examining the efficacy of the anti-PNAG antibody have been halted. However, trials on the antibody-antibiotic conjugate DSTA4637S [Genentech, San Francisco CA] against a narrower teichoic acid motif shows promise as an anti-*Staphylococcus* therapy (46).

Right Target, Wrong Time?

When designing antibodies *in vitro* for *in vivo* use, it is important not to overlook how targets differ in expression patterns in different disease contexts. In one notable example, mAb candidate KB001-A against *P. aeruginosa* Type III Secretion System (T3SS) protein *PcrV* successfully reduced pneumoniae incidence in mechanically ventilated patients colonized with Pa (47), but failed to reduce the need for antibiotics in Pa colonized cystic-fibrosis patients (17). Possible reasons for this disparity include observations that T3SS is reduced in expression in *Pseudomonas* isolates that colonized CF patients, including those of the same clonal background (48). As a result, more investigation is warranted to evaluate expression of virulence factors under different natural disease conditions to better predict the therapeutic benefits of these targets. This is also relevant when examining different infection sites; *Enterobacteraciae* colonizing the urinary tract may increase presentation of fimbriae, while in the blood they may prefer production of capsular polysaccharides (49, 50). Additionally, some ubiquitous proteins may in some contexts become virulence factors worthwhile to target, such as DNA-binding proteins in the formation of biofilms (51)*.

Examining the immunome may provide a unique perspective of this challenge, as recent work has demonstrated that patients who recovered from different sites of *S. aureus* infection had IgG profiles that differed in what antigens they recognized (52)**. This high-throughput approach, as well as other surveys that examine immune activity against single antigens across various patient parameters (53), will yield a better understanding of disease-specific pathogen-host responses and help drive searches for the most potent target as well as select optimal patient populations in developing appropriate clinical trials.

Beyond the Variable Region: Different Antibody Activities and Development Strategies

In addition to studying the ideal target for each infection, understanding the ideal mechanisms of protection against each type of infection is paramount. Unlike an antibiotic or small peptide, whose action is simply to bind and modulate a target, antibodies possess a plethora of other capabilities due to its Fc region, including OPK (22, 35), agglutination, and complement interaction (20). Numerous studies have compared the importance of these capabilities in providing protection, and studied how differences in antibody subclass

differences and other FcR structural factors can affect them. Notably, work studying differences between opsonic and non-opsonic antibodies against *Streptococcus pneumoniae* has shown that non-opsonic antibodies could alternatively module gene expression to force capsule shedding of the bacteria, decrease iron acquisition, and increase susceptibility to oxidative stress (37). Such expression-altering abilities and direct bactericidal mechanisms beforementioned (31) will inspire work to explore these new exciting mechanisms of action, which could be particularly relevant in the treatment of infections in immunocompromised patients. These patients are most susceptible to AMR organisms, and their potential gain from mAb therapy could be highest. Most AMR isolates, including CR-*Kp* have low virulence characteristics and are easily killed by immunocompetent serum (36, 41). Unfortunately, the low virulence of these bacteria creates the challenge of designing adequate animal models to study infection. While we and other colleagues have had successes in generating several antibodies against CR-*Kp* (35, 36, 41), these antibodies have been difficult to test *in vivo* due to the bacteria's inability to cause significant disease in the tested animal models absent high inoculums or sensitization (41).

Additionally, new technologies and innovations continue to improve mAb design. High throughput strategies that have advanced from phage display to the use of FACS to quickly isolate native cells that bind the investigator's target of choice have replace laborious hybridoma screenings (30, 31, 40). Structural analyses have revealed insights into specific differences between antibody subclasses and accelerated efforts to equip mAbs with artificial mechanisms of action (46, 54). Additionally, studies to reduce the size of mAb-based molecules have improved penetration into infection foci and have allowed endogenous production in food to limit transmission.

CONCLUSION

The field of anti-infective mAbs is progressing with leaps and bounds as researchers are finding potent targets, acquiring more in-depth understanding of the expression and roles of target antigens with respect to disease pathogenesis, and making technological advances in developing and screening mAbs. Despite this progress, this field must still overcome major hurdles to advance more antibodies to human therapy. Focus should be on establishing clinically-relevant in vitro correlates and animal models to improve correlation of preclinical and clinical study results. In addition, antigen heterogeneity in combination with the inherent dynamics of newly emerging clones has to be addressed as it continues to deter for pharmaceutical industries from investing. Recent successes suggest that mAbs could emerge as primary therapies against MDR pathogens whereas antibiotics will serve as adjuvant in such treatment plans.

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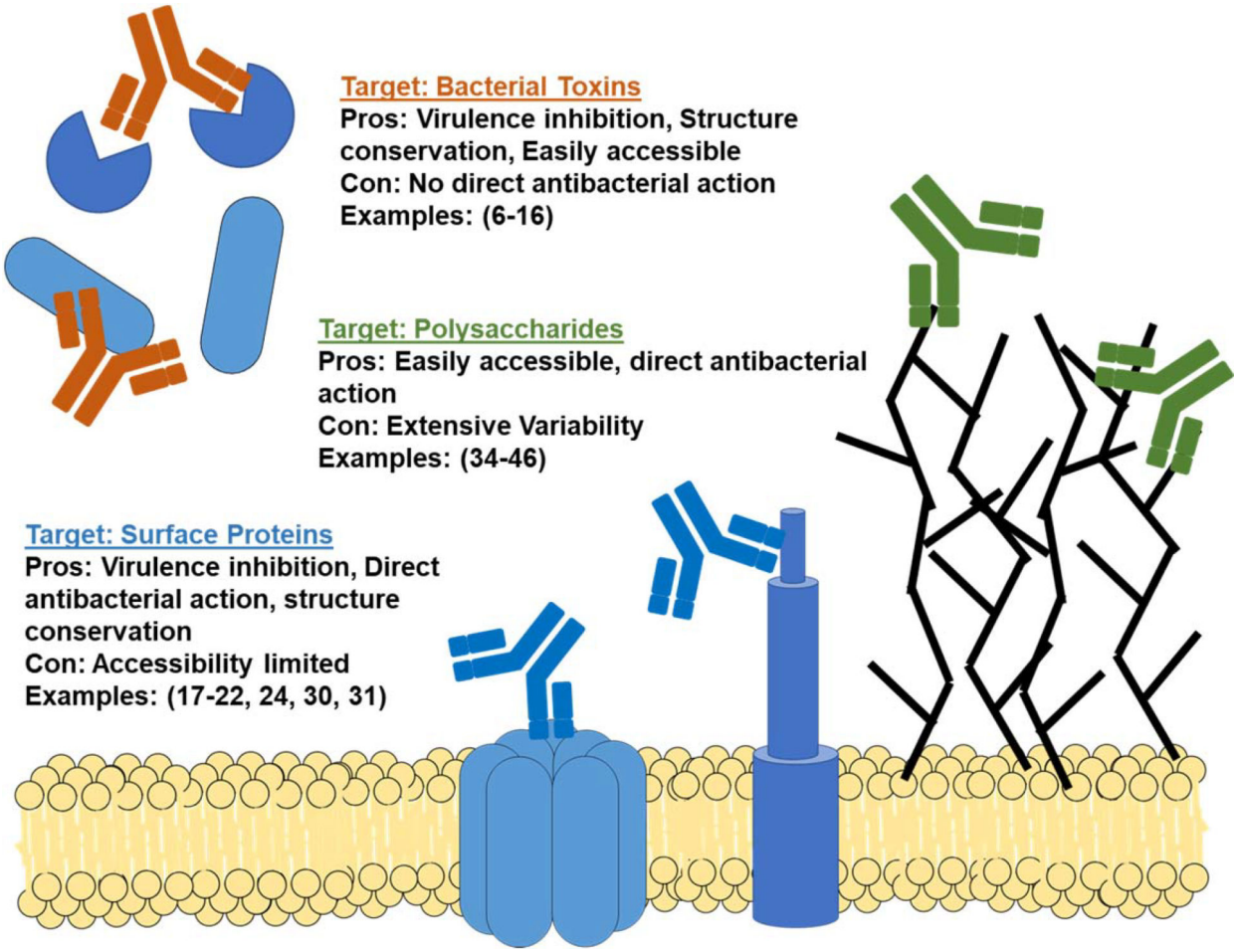

Figure 1:
Targets of antibody therapy against bacterial pathogens

Table 1:

Clinical Trials of new anti-bacterial antibody candidates updated since 2016 *Excludes studies of already licensed antibodies

Target	Name	Study Phase	Pathogen	Status	Trial Number
Toxin (Hla)	MEDI4892 (Suvratoxumab)	2	*S. aureus*	In progress (Enrollment Completed)	
Toxin (Hla)	AR-301	3	*S. aureus*	In preparation (Not Yet Recruiting)	
Toxin (Hla)	AR-301	1,2	*S. aureus*	Completed, Successful[a]	
Toxin (Multiple)	ASN100 [2 mAb Cocktail]	2	*S. aureus*	Terminated[b]	
Surface Protein (SpA)	514G3	1,2	*S. aureus*	Completed, Successful[c]	
Surface Protein (Flagellin)	PsAer [IgY Polyclonal] (Mukoviszidos)	3	*P. aeruginosa*	Completed, Results Unknown	
Polysaccharide (Psl) + Surface Protein (PcrV)	MEDI3902	2	*P. aeruginosa*	In progress (Recruiting)	
Polysaccharide (Alginate)	AR-105 (Aerucin)	2	*P. aeruginosa*	In progress (Recruiting)	
Polysaccharide (Teichoic Acid)	DSTA4637 (Antibody-Antbiotic Conjugate)	1b	*S. aureus*	In progress (Recruiting)	
Polysaccharide (PNAG)	F598	2	*S. aureus*	Terminated	

[a]Based on June 2017 Press Release: https://aridispharma.com/2017/aridis-pharmaceuticals-inc-presents-positive-phase-2a-safety-and-efficacy-data-of-salvecintm-ar-301-in-patients-with-severe-pneumonia-caused-by-staphylococcus-aureus-during-the-2017-asm-microbe-co/

[b]Based on June 2018 Press Release: http://investors.arsanis.com/news-releases/news-release-details/arsanis-provides-update-following-completion-planned-interim

[c]Based on April 2017 Press Release: http://investors.xbiotech.com/phoenix.zhtml?c=253990&p=irol-newsArticle&ID=2259222