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Review

Recent Advances in Immunotherapeutic and Vaccine-Based Approaches for the Treatment of Drug-Resistant Bacterial Infections

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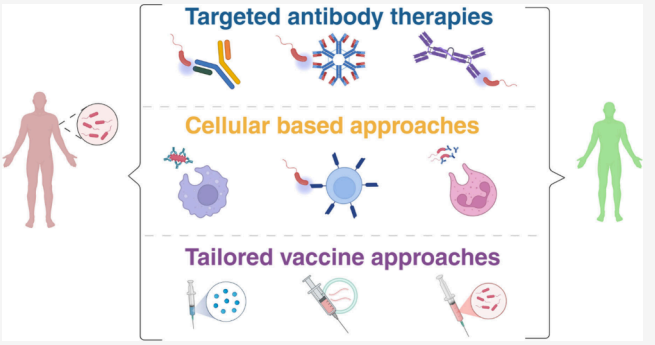
ABSTRACT: Antimicrobial resistance poses a grave threat to global public health. Although new antibiotics are urgently needed, most share resistance mechanisms with existing drugs, thereby necessitating the development of alternative antibacterial therapeutics. Various immunotherapeutic agents, including monoclonal antibodies, therapeutic vaccines, cellular therapies, and immunomodulators, have been developed and explored to treat drug-resistant bacterial infections. This review comprehensively summarizes recent advancements in immunotherapies and vaccine-based approaches as alternative strategies to combat drug-resistant bacterial infections. Our findings indicate that immunotherapy offers several advantages over traditional antibiotics, such as enhanced specificity, long-term effects, overcoming resistance mechanisms, broad applicability, potential for combination therapies, personalized medicine, and reduced toxicity. Also, formulation and delivery strategies, including nanoparticles, liposomes, cellular vehicles, and diverse administration routes, have been employed to improve the efficacy and targeting of these immunotherapeutic agents. In-depth evaluations of promising preclinical and clinical studies demonstrate their potential effectiveness against pathogens such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Mycobacterium tuberculosis*, *Streptococcus pneumoniae*, methicillin-resistant *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Helicobacter pylori*. These suggest that immunotherapy is a promising alternative to address the growing challenge of drug-resistant bacterial infections, potentially revolutionizing infection management strategies.



KEYWORDS: *immunotherapy, drug-resistant bacteria, monoclonal antibodies (mAbs), vaccines, adoptive cell therapy, immunomodulators*

INTRODUCTION

The development of antibiotic agents represents a monumental achievement in modern medicine.[1] However, the rampant and improper use of these agents has given rise to the emergence of multidrug-resistant (MDR) bacterial pathogens, which are commonly referred to as "superbugs".[2] The prevalence of antimicrobial resistance (AMR) has steadily increased over several decades, fuelling an epidemic of infections that evade conventional therapies.[3] Currently, superbugs are estimated to be responsible for approximately 700,000 fatalities worldwide each year, with projections indicating a staggering increase to 10 million by 2050.[4] This crisis predominantly revolves around the "ESKAPE" bacteria, including *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella* spp., *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.[5] The significance of ESKAPE pathogens lies in their association with severe infections in hospitals, particularly in intensive care units (ICUs), and their direct relationship with high mortality rates, particularly in children and elderly individuals.[6] These pathogens can cause various severe and potentially life-threatening conditions, ranging from skin and soft tissue infections to pneumonia, sepsis, and cystic fibrosis.[7]

Fortunately, there have been significant advancements in disease treatment through immunotherapies, which specifically target the host immune system.[8] Immunotherapy has revolutionized cancer treatment by reversing immunosuppression.[9] Interestingly, cancer and bacterial infections share common characteristics of immunosuppression,[10] suggesting that immunotherapy holds tremendous potential for treating bacterial infections, including persistent and drug-resistant infections.

Vaccines, as a preventive measure, have played a crucial role in combating both infectious diseases and AMR,[11] with formulations categorized based on their antigen type and delivery.[12] Live attenuated vaccines use weakened pathogens

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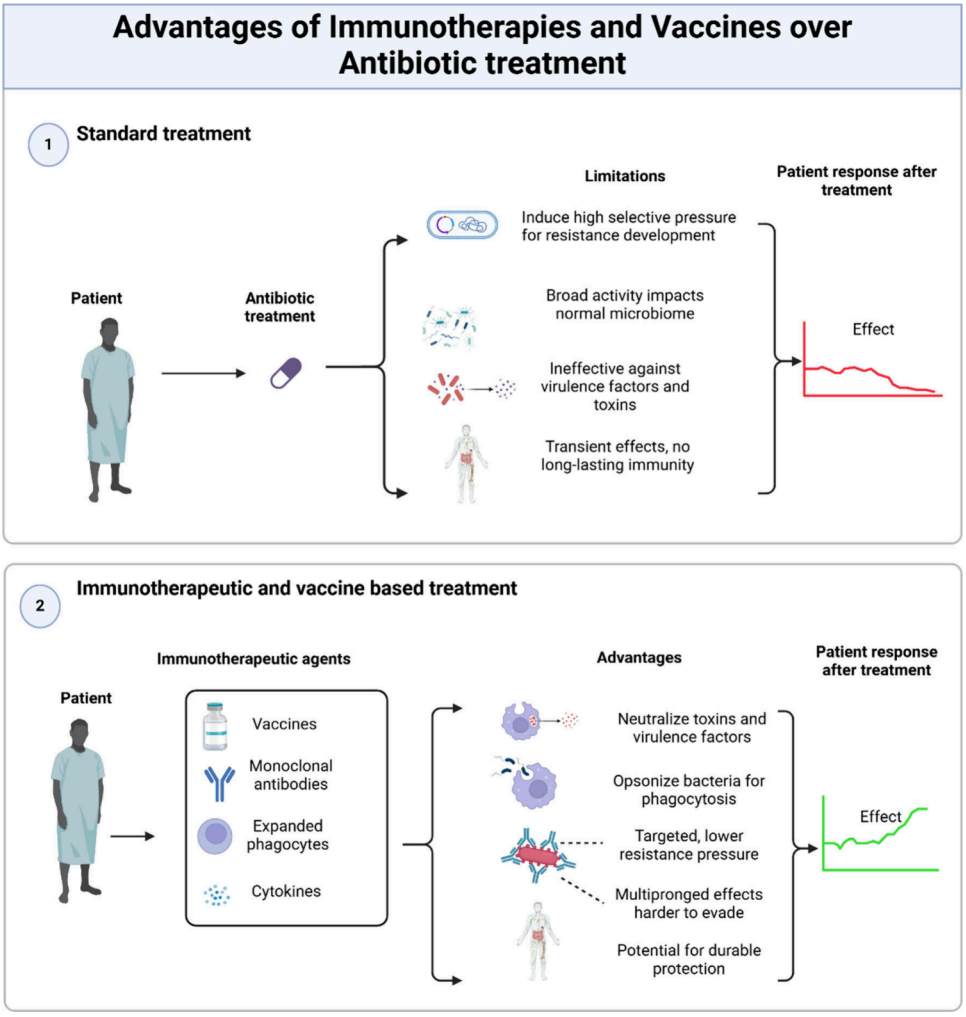

Figure 1. Comparison of antibiotic drawbacks with immunotherapy and vaccine benefits. On the upper panel of the diagram (1), the major drawbacks of antibiotics are depicted, including the widespread emergence of antibiotic resistance, the narrow spectrum of activity, the potential for disrupting the host microbiome, and the need for prolonged, repeated dosing without lasting immunity. In contrast, the lower panel of the diagram (2) emphsizes the significant benefits of immunotherapeutic and vaccine-based strategies. Immunotherapies target virulence factors, toxins, and other essential pathogenic determinants, eliciting diverse immune responses to overcome resistance and establish long-term protective immunity against specific bacterial pathogens.

that replicate and induce strong, long-lasting immunity but are unsuitable for immunocompromised individuals. Inactivated vaccines contain killed pathogens, offering safety but often requiring booster doses to maintain immunity. Subunit vaccines use specific pathogen components, such as proteins and polysaccharides, ensuring safety and reducing adverse reactions. Nonetheless, they elicit a weaker immune response compared to live attenuated vaccines. Conjugate vaccines, which link polysaccharide antigens to carrier proteins, have been particularly effectively protected against bacterial infections in children. More recently, advancements in vaccine technology have led to the development of DNA vaccines, mRNA vaccines, and viral vector vaccines, which harness the body's cellular machinery to induce an immune response. These vaccines have shown high efficacy and can be rapidly developed and manufactured.[12] The selection of the appropriate vaccine formulation depends on the target pathogen, the desired immune response, and the specific characteristics of the vaccine platform.[13] Ongoing research in vaccine development aims to improve the effectiveness, safety, and broader coverage against multiple strains or pathogens.

This Review focuses on recent advancements in the field of immunotherapies, which include a diverse range of treatments, such as vaccines, cellular-based therapies, antibody-based therapies, and immunomodulatory approaches. The aim is to provide a comprehensive summary of the various types of immunotherapies, emerging trends, ongoing and successful clinical trials, and potential future developments in this field.

RATIONALE FOR EXPLORING IMMUNOTHERAPY APPROACHES AS ALTERNATIVE STRATEGIES

Despite ongoing efforts to develop new antimicrobial agents, many share modes of action similar to those of existing drugs, inevitably leading to drug tolerance. Furthermore, over the past decade, there has been an immense and ongoing decrease in the supply of approved antibiotics. To address the challenges commonly encountered in managing and controlling AMR, such as limited effectiveness, toxicity, and the development of drug resistance, novel therapeutic solutions are necessary. Figure 1 presents a comparative overview of the limitations associated with conventional antibiotics and the key advantages of immunotherapies and vaccines as alternative approaches to

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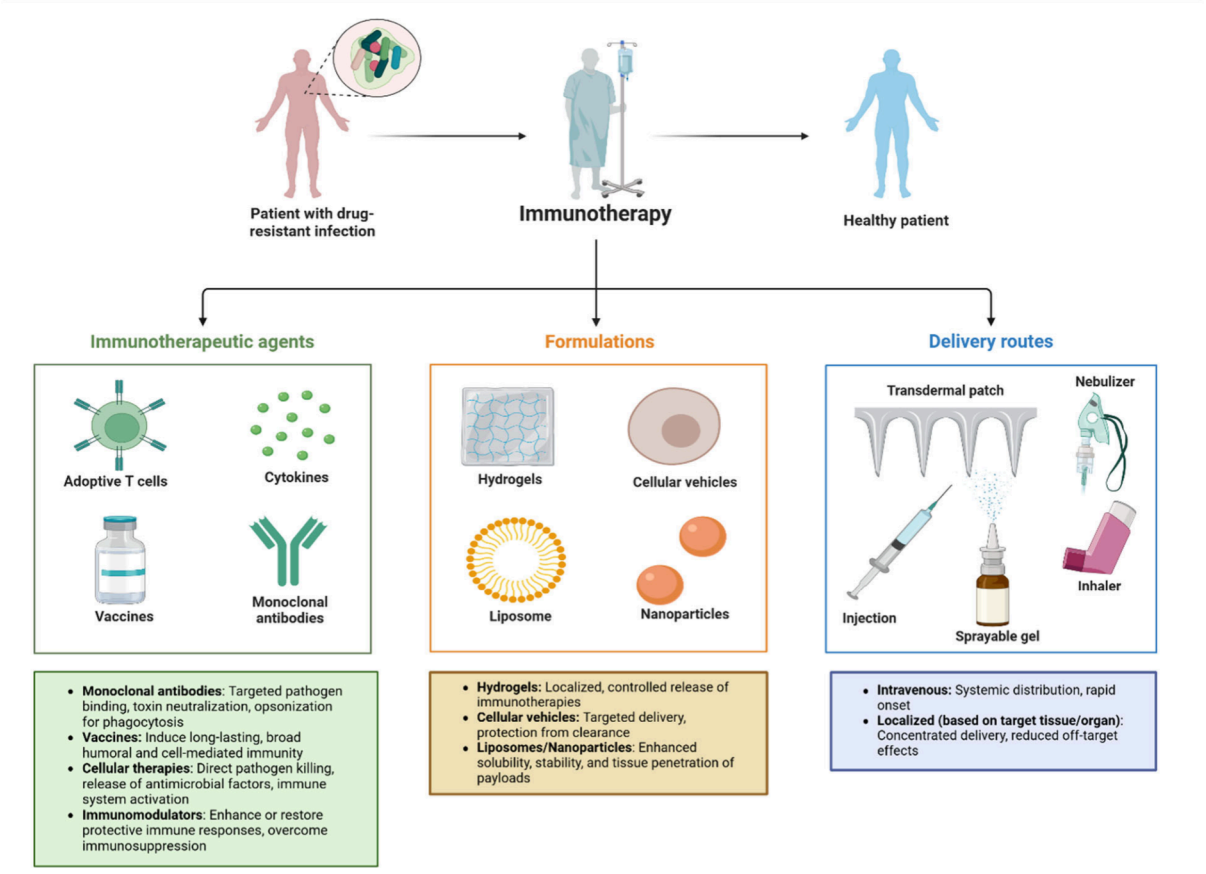

Figure 2. Diverse approaches to immunotherapy. The major categories of immunotherapeutic agents used to modulate immune responses include monoclonal antibodies, adoptive cell therapies, cytokines, and vaccines. Formulations such as hydrogels, cellular vehicles, liposomes, and nanoparticles are used to transport these immunotherapeutic agents to the infection site. Immunotherapeutic agents are delivered through various routes, from intravenous delivery to localized therapy, depending on the target tissue or organ.

combat drug-resistant bacterial infections. The exploration of immunotherapy approaches as alternative strategies for disease treatment has recently attracted significant interest and momentum. Several key rationales support this exploration:

(a) **Enhanced specificity:** Immunotherapy can target specific cells or molecules involved in disease processes. By harnessing the host's immune system, immunotherapeutic agents can be designed to identify and engage disease-related targets precisely, thereby reducing off-target effects and minimizing damage to healthy tissues.

(b) **Long-term effects:** Immunotherapy induces long-term responses by training the immune system to recognize and remember disease-specific signals. This memory response provides durable protection against disease recurrence or progression, offering potential long-term benefits to patients.

(c) **Overcoming resistance:** Immunotherapy approaches, particularly immune checkpoint inhibitors and cell-based therapies, can overcome certain resistance mechanisms by reactivating or enhancing the immune response, potentially providing an alternative when other treatments fail.

(d) **Broad applicability:** Immunotherapy has promise for treating various diseases and conditions. It is effective in treating several types of cancer, including melanoma, lung cancer, and hematological malignancies. In addition, immunotherapy is being explored for treating autoimmune disorders, infectious diseases, and even neurodegenerative conditions, expanding the potential applications of this approach.

(e) **Combination potential:** Immunotherapy can be combined with other treatment modalities, such as chemotherapy, radiation therapy, and targeted therapy. In addition, combinations of different immunotherapeutic agents enhance therapeutic efficacy through synergistic interactions.

(f) **Personalised medicine:** Immunotherapy allows a personalized approach to treatment. By analyzing the patient's immune profile and infection characteristics, therapies can be tailored to individual patients, potentially increasing the likelihood of positive responses and minimizing unnecessary treatments.

(g) **Reduced toxicity:** Compared with traditional therapies, immunotherapy offers a favorable safety profile with reduced toxicity. Although side effects can occur, they often differ from those associated with chemotherapy or radiation therapy. This can lead to an improved quality of life during treatment (Figure 1).

PRINCIPLES OF IMMUNOTHERAPY

The underlying principles of immunotherapy involve modulation or enhancement of the immune response to achieve therapeutic effects. Various immunotherapeutic agents, formulations, and delivery routes have been developed to enhance the efficacy and precision of immunotherapy (Figure 2).

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Table 1. Vaccine-Based Therapies Targeting ESAKAPE Pathogens and Their Virulence Factors

vaccine type	target species	target (virulence factors, proteins and antigens)	mechanism of action	development stage	refs
live attenuated	*Pseudomonas aeruginosa*	live attenuated KBMA strain	elicits both antibody and cellular immune responses	preclinical	15
	Escherichia coli K1	live attenuated ΔaroA mutant	generates humoral immunity and maternal antibodies for passive protection	preclinical	16
	Mycobacterium tuberculosis	heat-killed Mycobacterium indicus pranii (MIP)	enhances antigen-specific T cell responses and IFN-γ production	clinical	17
subunit/antigen-based	*Pseudomonas aeruginosa*	FpvA	elicits systemic and mucosal immunity; targets virulence factors	preclinical	18
	group B Streptococcus	α-C and Rib proteins	generates IgG antibodies that cross the placenta for passive protection	clinical	21
	Streptococcus pneumoniae	PspA and PhtD	induces robust antibody and cytokine responses; enhances complement-mediated bactericidal activity	preclinical	22
	Staphylococcus aureus	PBP2a and autolysin proteins	elicits opsonic antibodies and provides protection against lethal MRSA challenge	preclinical	38
	Acinetobacter baumannii	TrxA, OmpA, NlpE, ExeM/NucH, ZnuD, and TonB	targets multiple virulence factors to provide broad protection	preclinical	24
	Helicobacter pylori	urease, CagA, HopE, SabA, and BabA	focuses on conserved virulence factors to generate cross-protective immunity	preclinical	25
	Haemophilus influenzae	Tbp1 antigen	elicits robust antibody responses and provides protection against *Haemophilus influenzae*	preclinical	26
	Mycobacterium tuberculosis	Ag85b and ESAT-6	induces potent cellular and humoral immunity, comparable to the BCG vaccine	preclinical	27
mrna/nucleic acid	*Pseudomonas aeruginosa*	PcrV, OprI, and OprF	elicits robust cellular and humoral immune responses	preclinical	28
	Clostridioides difficile	TcdB, TcdA, and SlpA	induces broad and potent immune responses against multiple antigens	clinical	30
	Acinetobacter baumannii	AmpD, OmpA, Pal, BauA, Omp34, BamA, Omp22, CsuA/B, OmpK, and DcaP	enhances immunogenicity and protective efficacy compared with protein vaccines	preclinical	31
	Helicobacter pylori	LeoA	elicits Th2-dominant immune response and inhibits *H. pylori* growth	preclinical	32
Vaccine-based immunotherapy	*Escherichia coli*	TolC	inhibits efflux pump activity and restores antibiotic susceptibility	preclinical	34
	Staphylococcus epidermidis	SesC,	disrupts biofilm formation and enhances antibiotic efficacy	preclinical	35
	Staphylococcus aureus	SasG	disrupts biofilm formation and enhances antibiotic efficacy	preclinical	36
	Haemophilus influenzae	PilA	disrupts biofilm formation and enhances antibiotic efficacy	preclinical	37

Immunotherapeutic Agents. Immunotherapeutic agents can be categorized into different types, including monoclonal antibodies, therapeutic vaccines, cellular therapies, and immunomodulators. Monoclonal antibodies (mAbs) are laboratory-produced proteins that target specific antigens on diseased cells or molecules involved in disease processes. Therapeutic vaccines comprise disease-specific antigens or immune-stimulating molecules that promote an immune response to the targeted disease. Cellular therapies are modified cells that can specifically recognize and eliminate infected cells, thereby enhancing the body's natural immune response. Immunomodulators are signaling molecules that regulate immune responses to enhance disease-fighting capabilities (Figure 2).

Formulations. Formulation is critical for the delivery of immunotherapeutic agents. Different formulations, including nanoparticles, liposomes, cellular vehicles, and conjugates, are used to improve drug stability, enhance targeting, prolong circulation time, and control the release of immunotherapeutic agents. These formulations ensure optimal efficacy while minimizing side effects (Figure 2).

Delivery Routes. The delivery route for immunotherapy depends on the specific agent and disease. Intravenous (IV) infusion allows for the systemic distribution of immunotherapeutic agents throughout the body, whereas subcutaneous (SC) or intramuscular (IM) injections provide localized delivery (Figure 2).

IMMUNOTHERAPY FOR DRUG-RESISTANT BACTERIAL INFECTIONS

Advances have been made in immunotherapeutic and vaccine-based approaches against antibiotic-resistant ESKAPE bacterial pathogens. Below, we present recent progress in four key categories of immunotherapy against specific ESKAPE bacterial infections. Tables 1−3 summarize promising preclinical/clinical trials.

VACCINE-BASED IMMUNOTHERAPY

Vaccines are broadly divided into two categories: live and nonlive. Live vaccines are made from a weakened or attenuated form of the pathogen that retains the ability to replicate within the body but is significantly weakened compared to the wild type. Conversely, nonlive vaccines use either inactivated (killed) forms of the pathogen or purified components, such as proteins and polysaccharides, to stimulate an immune response. Live vaccines are designed to mimic natural infections, enabling the immune system to mount a strong and lasting immune response. However, these treatments are not recommended for individuals with a weakened immune system because of their potential risks. Nonlive vaccines are considered safer for individuals with

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Table 2. Antibody-Based Therapies Targeting Bacterial Pathogens and Their Virulence Factors

antibody type	target species	target	mechanism of action	development stage	refs
monoclonal antibodies (mAbs)	*Klebsiella pneumoniae*	O2 antigen	neutralization, opsonization, complement activation	preclinical	45
	Klebsiella pneumoniae	Type 3 fimbriae (MrkA)	neutralization, opsonization, complement activation	preclinical	48
	Staphylococcus aureus	SpA	inhibition of virulence factors	clinical	66
	Staphylococcus aureus	PBP2a	inhibition of virulence factors	preclinical	49
	Pseudomonas aeruginosa	O-antigen	neutralization, opsonization	preclinical	50
	Pseudomonas aeruginosa	Flagellin	complement-mediated bactericidal effects and improved opsonophagocytosis	preclinical	51
	Pseudomonas aeruginosa	PcrV, Psl	opsonization, enhancement of phagocytosis, protection against infection	preclinical/ clinical	53
	Acinetobacter baumannii	ATP synthase	opsonization, enhancement of phagocytosis, protection against infection	preclinical	54
	Clostridium difficile	Toxin B	neutralization of *Clostridium difficile* toxin B	clinical	57
antibody engineering and delivery	*Staphylococcus aureus*	Enterotoxin B	continuous antibody secretion, enhanced pharmacokinetics	preclinical	65
	Staphylococcus aureus	Protein A	overcoming staphylococcal protein A-mediated immune evasion	clinical	66
	Pseudomonas aeruginosa	PcrV, Psl	targeting multiple virulence factors for enhanced protection	preclinical	78
	Pseudomonas aeruginosa	PcrV, Psl	long-lasting *in vivo* antibody production	preclinical	67
antibody targeting resistance mechanisms	Gram-negative bacteria	β-lactamases	inhibition of antibiotic-inactivating enzymes (e.g., β-lactamases)	preclinical	68
	Pseudomonas aeruginosa	Alginate	disruption of biofilm structure, enhancement of antibiotic efficacy	preclinical	70

weakened immune systems because they do not pose a risk of developing the targeted disease. Another vaccine classification distinguishes between cell- and antigen-based vaccines, focusing on the type of component used to stimulate the immune response. Cell-based vaccines utilize whole cells of the pathogen, which can be live, inactivated (killed), or attenuated (weakened). In contrast, antigen-based vaccines target specific pathogen components or antigens. These vaccines use purified fragments, proteins, polysaccharides, or other antigenic molecules derived from the pathogen.[14]

Vaccines present the immune system with an attenuated or inactive form of a pathogen or its components, stimulating an adaptive immune response. Vaccines can target bacterial antigens, including cell surface polysaccharides, proteins, and toxins, to elicit a protective immune response.[14] This response involves the production of antibodies and the activation of T cells, which can recognize and eliminate the pathogen upon subsequent exposure. Vaccines can provide long-lasting protection by creating immunological memory, which enables the immune system to respond rapidly and effectively upon reinfection.

Research, Case Studies, Preclinical and Clinical Trials. *Live and Attenuated Vaccines.* Live attenuated vaccines show greater promise by eliciting both antibody and cellular immune responses essential for protection. Building on this strategy, a novel live attenuated *Pseudomonas aeruginosa* vaccine was developed using the killed but metabolically active (KBMA) attenuation method, offering enhanced safety while maintaining immunogenicity.[15] The KBMA-engineered strain was designed to overexpress key virulence factors, including the type III secretion system, while maintaining attenuation. Mice vaccinated with KBMA *P. aeruginosa* developed robust humoral immunity against key antigens such as the PcrV and OprF proteins. Analysis of serum cytokine levels revealed that the vaccine stimulated Th1, Th2, and, notably, Th17 cellular responses. Most significantly, the KBMA *P. aeruginosa* vaccine was safe and provided a protective efficacy in a challenging pulmonary infection model.

Preterm birth significantly contributes to neonatal morbidity and mortality, with preterm infants being especially vulnerable to severe bacterial infections, particularly those caused by *Escherichia coli*, including the virulent *E. coli* K1 strains. Women with a history of preterm deliveries are at an elevated risk of recurrence, making them a key target group for a vaccine aimed at preventing *E. coli* neonatal infections. Recent research has developed a live attenuated *E. coli* K1 E11 strain through targeted deletion of the virulence-associated aroA gene.[16] The ΔaroA mutant demonstrated reduced adhesion and invasion of epithelial cells along with decreased expression of type 1 fimbriae, a key virulence factor in *E. coli* K1. *In vivo* experiments indicated that the ΔaroA mutant was significantly less infectious than the wild-type strain, indicating its potential as a safe live vaccine candidate. Immunising adult female mice with the *E. coli* K1 E11 ΔaroA vaccine resulted in a strong humoral immune response, characterized by high polyclonal bactericidal antibodies directed at both *E. coli* K1 and non-K1 strains. These vaccine-induced antibodies conferred substantial protection against lethal challenges from multiple *E. coli* strains in adult mice. Notably, maternal antibodies produced by the vaccine were effectively transmitted to the offspring, providing the mouse pups with strong protection against severe *E. coli* infections, including meningitis. This study provides compelling preclinical evidence for developing a live attenuated *E. coli* K1 vaccine to protect pregnant women and their newborns from severe *E. coli* infections, particularly meningitis.

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Table 3. Summary of the Different Types of Immunotherapies, Ongoing and Successful Clinical Trials

immunotherapeutic approach	type	description	targeted bacteria	clinical outcome	stage	refs
			Antibody-based			
	monoclonal antibodies	*P. aeruginosa* cultured on ammonium metavanadate	*P. aeruginosa*	decreased generation of inflammatory cytokines and bacterial burden	preclinical	50
	monoclonal antibodies	Fc-engineered version with Q311R/M428E/N434W (REW)	*N. gonorrhea, S. pneumonia, S. aureus*	eradication of both Gram-positive and Gram-negative bacteria	preclinical	64
	monoclonal antibodies	Pse-MAB1 targets pseudoaminic acid (Pse)	*A. baumannii*	bactericidal activity against *A. baumannii*	preclinical	56
	antibody–drug conjugate (ADC)	designed ADC by fusion of an antimicrobial peptide at the C-terminus of VSX antibody's VH or VL chains	*P. aeruginosa*	eradicated strains of *P. aeruginosa*	preclinical	52
	monoclonal antibodies	anti-O2 antibodies combined with Meropenem	*K. pneumoniae*	synergistically defends against drug-resistant strains	preclinical/ clinical	45
	polyclonal antibodies	anti-BCAL2645 goat polyclonal antibody binds to OmpA-like proteins	*P. aeruginosa*	reduction in biofilm formation	preclinical	59
	mammalian monoclonal antibodies	mAbs that target the type 3 fimbrial (T3F) protein MrkA	*K. pneumoniae*	promoted killing of the acapsular *K. pneumoniae* strain	preclinical	48
	mammalian monoclonal antibodies	monoclonal antibody targeting the penicillin-binding protein 2a (PBP2a)	*MRSA*	strong binding affinity to both the recombinant and native forms of PBP2a	preclinical	49
	bispecific monoclonal antibody	a bispecific monoclonal antibody targeting two key virulence factors of *Pseudomonas aeruginosa*—Psl and PcrV	*Pseudomonas aeruginosa*	improved neutrophil phagocytosis and killing of *P. aeruginosa*	preclinical	53
	avian immunoglobulin Y (IgY) antibodies	anti-DEC-IgY against diarrheagenic *Escherichia coli* infections	*E. coli*	reduced colonization in the intestine and mitigated infection severity	preclinical	60
	avian immunoglobulin Y (IgY) antibodies	chicken-derived antibodies targeting the recombinant *S. pneumoniae* Eno1 (spEno1) protein	*S. pneumoniae*	exhibited strong binding activity to spEno1	preclinical	61
	polyclonal avian antibodies (IgY)	polyclonal avian antibodies (IgY) raised against inactivated whole cells of *Pseudomonas aeruginosa* (PA) PAO1 strain	*Pseudomonas aeruginosa*	enhanced the opsonophagocytic killing of PA by polymorphonuclear leukocytes	preclinical	62
	avian immunoglobulin Y (IgY) antibodies	IgY antibodies targeting a chimeric recombinant protein that incorporates three major virulence factors of *Vibrio cholerae*: OmpW, TcpA, and CtxB.	*Vibrio cholerae*	the anti-OTC IgY effectively neutralized the cytotoxic effects of cholera toxin	preclinical	63
	monoclonal antibodies	adeno-associated virus (AAV) vectored immunoprophylaxis	*P. aeruginosa*	provided significant protection against lethal intranasal challenges	preclinical	67
			Vaccine-based			
	passive immunization (maternal vaccination)	GBS-NN/NN2 (modified vaccine candidate)	group B Streptococcus (GBS)	protect newborns through maternal immunization during pregnancy	preclinical	21
	active immunization (vaccine)	absorbance of α-C and Rib on GBS	GBS	reduced recovery of GBS in systemically challenged vaccinated mice	preclinical	20
	conjugate nanovaccine	PLGA-based nanoparticle formulation of recombinant PBP2a and MRSA autolysin	MRSA	decreased mortality rate and complete removal of MRSA from the kidneys of infected mice	preclinical	38
	peptide-based subunit vaccines	conjugated FpvA peptides with Curdlan as adjuvant to keyhole limpet hemocyanin (KLH)	*P. aeruginosa*	reduced pulmonary edema and bacterial load following *P. aeruginosa* challenge	preclinical	18
	live and attenuated vaccine	killed but metabolically active (KBMA) attenuation	*P. aeruginosa*	protective efficacy in a novel pulmonary infection model	preclinical	15
	fusion peptide vaccine	The PspA and PhtD antigen immunodominant regions are combined into a single construct (PAD)	*S. pneumoniae*	high levels of cytokines and antibodies produced in immunized mice, and bactericidal activity	preclinical	22
	live and attenuated vaccines	live attenuated *E. coli* K1 E11 ΔaroA strain by deleting the aroA gene	*E. coli*	induced a strong humoral immune response	preclinical	16
	subunit and antigen-based vaccines	macrophage membrane-coated nanoparticles	*P. aeruginosa*	exhibited robust humoral immune responses	preclinical	19
	subunit and antigen-based vaccines	multiepitope construct encapsulated within lipid nanoparticles	*H. pylori*	cross-protective immunity	preclinical	25

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Table 3. continued

immunotherapeutic approach	type	description	targeted bacteria	clinical outcome	stage	refs
	subunit and antigen-based vaccines	derived from the highly conserved tbp1 (transferrin-binding protein 1) antigen of *Haemophilus influenzae*	*Haemophilus influenzae*	significantly higher antibody responses	preclinical	26
	subunit and antigen-based vaccines	purified H1 antigen formulated with a liposomal adjuvant called NanoSTING,	*Mycobacterium tuberculosis* (M. tb)	elicited robust antigen-specific T cell responses	preclinical	27
	mRNA vaccine	mRNA vaccines encoding the PA virulence factors PcrV (type III secretion system) and a fusion of outer membrane proteins OprF and OprI	*P. aeruginosa*	elicited strong antigen-specific humoral and cellular immune responses	preclinical	28
	mRNA vaccine	multivalent mRNA-lipid nanoparticle (mRNA-LNP) vaccine	*C. difficile*	elicited robust antibody responses	preclinical	30
	mRNA vaccine	three multiepitope mRNA vaccine constructs (ABV1, ABV2, ABV3)	*A. baumannii*	eliciting protective immunity against *A. baumannii* infections	preclinical	31
	repRNA vaccine	replicating RNA (repRNA) vaccine platform, a fusion of four M. tb antigens	*Mycobacterium tuberculosis (M. tb)*	induces robust systemic antibody responses and some mucosal immunity	preclinical	29
			Adoptive cell therapy			
	macrophage	introduction of CAR genes and CASP11 shRNA into macrophages	*S. aureus*	targeted eradication of *S. aureus*	preclinical	82
	neutrophils	nanostructured lipid carriers (NLCs) internalized into neutrophils	MRSA	mitigated MRSA burden	preclinical	114
	neutrophils	neutrophil-targeted nanocarriers	MRSA	MRSA biofilm colonies were destroyed and the number of intracellular bacteria decreased	preclinical	87
	Vγ9 Vδ2 T cells	infusion of 1 × 108 cells every 2 weeks for 12 weeks, followed by a 6-month follow-up period	MDR-TB	Alleviation of *M. tuberculosis* load *in vivo*	clinical	79
	cytokine-induced killer (CIK) cells	eight courses of second-line anti-TB medication combined with CIK cell-based immunotherapy	MDR-TB	CIK immunotherapy in combination with second-line anti-TB regimens against MDR-TB	clinical	115
	cytokine-induced killer (CIK) cells	combination of CIK immunotherapy and anti-TB chemotherapy	MDR-TB	alleviated symptoms, improved lesion absorption, and increased recovery	clinical	80
	macrophage	transfer of macrophages loaded with Lyso700D as a photosensitizer for the photodynamic effect against MRSA and *Acinetobacter baumannii* (AB)	MRSA, *Acinetobacter baumannii* (AB)	efficient elimination of MRSA and AB bacteria and improved survival in infected mice	preclinical	83
	macrophage	CAR mRNA and CASP11 siRNA intracellularly to macrophages	MRSA	enhanced ability to phagocytose and digest MRSA intracellularly.	preclinical	81
	macrophage	macrophage-targeted nanodecoy system based on iron oxide nanoparticles (IONPs), termed IONPs-PAA-PEG-MAN	*Mycobacterium tuberculosis* (M. tb)	increased intracellular M. tb killing in infected and monocyte-derived macrophages.	preclinical	85
immunomodulatory approaches						
	lactomodulin	discoveries of lactomodulin, a special peptide with dual anti-inflammatory and antibacterial properties	MRSA, vancomycin-resistant *Enterococcus faecium* (VRE)	dual anti-inflammatory and antibiotic properties of lactomodulin and its efficacy against antibiotic-resistant strains (MRSA, VRE)	preclinical	90
	human, canine, and equine MSC	TLR-3-activated MSC with vancomycin	MRSA	reduced bacterial and inflammatory cytokine counts	preclinical	92
	neutrophils	loading neutrophils with azithromycin and colistin for targeted delivery and enhanced bactericidal activity	*P. aeruginosa*	provided mice with efficient defense against *P. aeruginosa* infection	preclinical	116
	neutrophils	function of CRAMP in the host defense against *Acinetobacter baumannii*	*Acinetobacter baumannii* (AB)	CRAMP enhances neutrophil antibacterial activity	preclinical	91
	macrophages	effect of Ch25h on the innate immune response to *S. pneumoniae*	*Streptococcus pneumoniae*	Ch25h regulates cytokine and chemokine production, enhanced phagocytosis, and bacterial clearance	preclinical	94
	MSC-derived conditioned medium (MSC-CM)	investigating the impact of MSC-CM on the antibacterial and immunomodulatory effects of *P. aeruginosa*-infected HCECs	*P. aeruginosa*	*P. aeruginosa* proliferation is inhibited by MSC-CM	preclinical	93

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A multicenter clinical trial evaluated *Mycobacterium indicus pranii* (MIP) as an immunotherapeutic adjunct to standard antitubercular treatment (ATT) in 890 sputum smear-positive category II pulmonary tuberculosis (PTB) patients.[17] Participants received either heat-killed MIP or a placebo alongside conventional therapy, with the primary outcomes measuring the time to sputum smear/culture conversion and the secondary outcomes assessing the 2 year cure and relapse rates. The MIP-treated group demonstrated significantly improved culture conversion rates compared with the controls at week 4 (67.1% vs 57%) and week 39 (94.2% vs 89.2%). The cure rates were 94.2% for the MIP group, slightly higher than the 90.4% observed in the placebo group; however, this difference was not statistically significant. Importantly, MIP was found to be safe with fewer adverse events and demonstrated enhanced efficacy in high-risk populations, achieving 100% cure rates in MDR (2−3 drug resistance) patients and notable improvements in those with high bacillary loads and bilateral cavitations. Immunological analyses revealed increased antigen-specific T cell proliferation and IFN-γ production in the MIP group. The safety profile and promising results, especially in the high-risk subgroups, make a strong case for further investigation of MIP as an adjunct to ATT. More extensive studies with culture-based end points and extended follow-up are needed to fully characterize the long-term clinical impact of this novel immunotherapeutic approach.

Subunit and Antigen-Based Vaccines. *P. aeruginosa* is an opportunistic bacterium that causes chronic or acute respiratory infections with serious repercussions for individuals, particularly those with cystic fibrosis. Outer membrane proteins (OMPs) are important vaccine candidates for *P. aeruginosa* because they are surface-exposed and conserved and can elicit an immune response. The two major OMPs under investigation are porin F (OprF) and lipoprotein I (OprI). Studies have also demonstrated the importance of including Th17-promoting adjuvants for vaccine efficacy. For example, Sen-Kilic and co-workers[18] developed a new subunit vaccine targeting the extracellular region of FpvA, a crucial protein involved in iron uptake, to generate an effective vaccine against *P. aeruginosa*. The adjuvant curdlan was added when peptides from this target were linked to keyhole limpet hemocyanin (KLH). Mice were administered intranasal vaccines containing the FpvA-KLH conjugate. Vaccinated mice had lower lung edema and bacterial burdens than unvaccinated controls after being challenged with *P. aeruginosa*. Immunization induced systemic and pulmonary antigen-specific IgG, IgM, and IgA antibody responses. Furthermore, it promoted the recruitment of resident memory T and dendritic cells to the lungs. Additional investigations showed that FpvA-KLH vaccination produced IL-17, suggesting a favorable Th17 immune response. The findings indicate that this peptide-based intranasal vaccine elicits both mucosal and systemic protection.

An innovative multiantigen nanovaccine was developed against *P. aeruginosa*, demonstrating a novel therapeutic approach.[19] The vaccine-coated nanoparticles incorporated various *P. aeruginosa* virulence factors, including ExoA, which were subsequently coated with macrophage membranes, resulting in macrophage membrane-coated nanoparticles. This biomimetic design allows the presentation of multiple vaccine targets while mimicking the immune response. *In vivo* and *in vitro* safety tests confirmed that the formulation was well tolerated. Mice administered the macrophage nanovaccine through different routes exhibited robust humoral immune responses. This result significantly translated into enhanced protection in a challenging pneumonia infection model. This work sheds new light on the development of effective and safe multitarget antiviral vaccines using biomimetic nanotechnology.

Group B *Streptococcus* (GBS) causes serious complications during pregnancy, including stillbirth, preterm labor, fetal injury, and neonatal infections. The GBS-NN vaccine, which combines specific proteins (α-C and Rib) from GBS, has been shown to be safe in nonpregnant women, but its effectiveness during pregnancy requires further investigation. The vaccine targets GBS α-like surface proteins to generate antibodies (IgG) that cross the placenta. This allows passive immunization of the baby in utero and protection for the first 3 months of life. To assess vaccine efficacy, mouse models were used to mimic the outcomes of human GBS infection. The results showed that vaccinated mice had increased levels of GBS-NN-specific antibodies and reduced bacterial recovery in systemic infections. Although the vaccination did not completely eradicate GBS during pregnancy, the vaccinated dams exhibited fewer miscarriages. Maternal immunization also increased neonatal survival after intranasal GBS exposure.[20] The GBS-NN vaccine is a fusion protein consisting only of the N-terminal domains of the α-C and Rib surface proteins but did not exhibit reactivity toward two other significant N-terminal proteins, Alp1 and Alp2/3. To enhance vaccine coverage, the GBS-NN formulation was modified to incorporate all four AlpN proteins, resulting in the improved GBS-NN/NN2 vaccine. A phase I trial verified that GBS-NN/NN2 was well tolerated and highly immunogenic in humans, and preclinical safety tests revealed no side effects. As the vaccine aims to protect newborns through maternal immunization during pregnancy, reproductive studies in rats and rabbits have established an appropriate safety margin, approximately 40× the projected human dose to support GBS-NN/NN2 testing in a clinical trial when administered during the second and third trimesters of pregnancy.[21]

Streptococcus pneumonia (pneumococcus) causes a significant disease burden worldwide, particularly in newborns and elderly individuals. The existing polysaccharide vaccines have limitations; therefore, the protein antigens involved in virulence hold promise for improved versions. Research has focused on developing a fusion vaccine construct (PAD) combining the immunodominant regions of two highly protective surface proteins, PspA (PA) and PhtD (PD), identified through immunoinformatics analysis.[22] Computational analyses predicted that PAD would be nontoxic, antigenic, and able to provoke strong immune responses. Mice were immunized with PAD alone or with PA/PD individually or in combination. These results confirmed that PAD systemically induced high antibody and cytokine levels. Antibodies mediate strong complement-dependent bactericidal activity *in vitro*. Most notably, PAD provided the best survival protection following the challenge. PAD outperformed the individual and combined formulations, highlighting its potential as a universal vaccine or conjugate vaccine component. This is the first report of a novel pneumococcal vaccine candidate strategically fusing these two protective antigens.

Methicillin-resistant *Staphylococcus aureus* (MRSA) causes serious and potentially life-threatening infections. A novel nanoparticle vaccine was developed to combat MRSA by conjugating recombinant surface proteins PBP2a and autolysin to poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles using chemical cross-linkers.[23] Mice inoculated with the r-PBP2a-r-autolysin-PLGA nanovaccine exhibited higher levels of opsonic

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antibodies and different IgG subclasses than those in the other groups. Significantly, vaccinated mice experienced a lower mortality after the MRSA challenge. The vaccine also cleared MRSA from the infected kidneys. This study demonstrated that the r-PBP2a-r-autolysin-PLGA nanovaccine is highly immunogenic and provides valuable protection against lethal MRSA doses in an animal model.

Acinetobacter baumannii is an opportunistic pathogen that can cause hospital-acquired infections, which are associated with high mortality rates and significant healthcare costs due to their virulence, persistence, and limited treatment possibilities. A recent study developed and evaluated an immunoinformatics-designed multipeptide vaccine against *A. baumannii*, incorporating five antigenic peptides derived from the virulence factors Omp38, NucAB, NlpE, TonB, and ZnuD.[24] These peptides were subsequently fused with *A. baumannii* thioredoxin A (TrxA) to create the multipeptide vaccine construct designated as AMEV2. Mice immunized with the rAMEV2 construct and the AddaS03 adjuvant elicited a robust humoral immune response, characterized by elevated levels of IgG1 and IgG2c antibodies against both the complete rAMEV2 construct and its individual peptide components. Furthermore, AMEV2 vaccination induced a Th2-biased T cell response, as evidenced by increased IL-4-secreting splenocyte counts after restimulation with rAMEV2 or UV-inactivated *A. baumannii*. Notably, mice vaccinated with rAMEV2 demonstrated a 60% survival rate following a lethal intranasal challenge with a hypervirulent strain of *A. baumannii*, alongside a significant reduction in bacterial burdens in the lungs, spleen, kidneys, and blood when compared with mock-vaccinated controls. The immunoinformatics-based design of the multipeptide AMEV2 construct represents a promising approach to creating safer and more effective subunit vaccines against MDR pathogens.

Helicobacter pylori is a common infection in humans and is associated with various chronic and acute gastric diseases as well as some extra-gastric disorders. Designing an effective *H. pylori* vaccine is challenging but crucial because current treatments are limited. A multiepitope vaccine against *H. pylori* was developed through the immunoinformatics-driven selection of five optimal epitopes from key virulence proteins (SabA, Urease, HopE, BabA, and CagA), followed by a lipid nanoparticle formulation.[25] The final multiepitope structure was created using the solid-phase technique and encapsulated in lipid nanoparticles with an average size of 154 nm and a spherical shape. Interestingly, the multiepitope vaccine construct demonstrated high MHC binding (99.05%), low toxicity, and nonallergenic properties, indicating its potential for safe and effective immunization. *In vitro* characterization showed a high loading efficiency of approximately 91% for the multiepitope construct within the lipid nanoparticles. The rationally designed multiepitope vaccine targeting key *H. pylori* virulence factors represents a promising approach to prevent and treat *H. pylori* infection.

Haemophilus influenza is a bacterium that causes respiratory infections, with type b (Hib) being known for causing severe illnesses, such as meningitis and sepsis, in children. Two synthetic peptide vaccine candidates derived from the highly conserved tbp1 (transferrin-binding protein 1) antigen of *Haemophilus influenzae* were validated through comprehensive *in vivo* testing in BALB/c mouse models.[26] The tbp1-E1 and tbp1-E2 peptides were selected based on previous *in silico* analyses that predicted their potential as T and B cell epitopes. These peptides were formulated using two different adjuvants (bacterial ghosts, BGs; and incomplete/complete Freund's adjuvant, IFA/CFA) and administered subcutaneously to BALB/c mice in a prime-boost regimen. An indirect ELISA was performed to evaluate the antibody titers against the peptides in the sera of the immunized mice. The results revealed that combining both tbp1 peptides with the BG adjuvant elicited the highest IgG antibody titers and absorbance values compared with the other groups. Statistical analysis confirmed that the antibody responses were significantly higher in the peptide-vaccinated groups than in the controls. This study provides a proof of concept demonstrating the *in vivo* efficacy of the synthetic tbp1 peptide-based vaccine, which could lead to the development of an effective cross-strain *H. influenzae*-based vaccine.

The development of potent tuberculosis (TB) vaccines continues to be a crucial focus in global health given that *Mycobacterium tuberculosis* (M. tb) remains a significant contributor to mortality on a global scale. A novel TB vaccine candidate was developed using a fusion protein (H1) combining *M. tuberculosis* antigens Ag85B and ESAT-6. The antigen was engineered with N-terminal Sumo tags to improve its solubility and expression, which was later removed by protease cleavage. When formulated with the cGAMP-containing NanoSTING liposomal adjuvant, the vaccine demonstrated enhanced antigen stability and prolonged nasal cavity retention compared to nonadjuvanted controls. Intranasal immunization of mice with the NanoSTING-H1 vaccine elicited robust antigen-specific T cell responses in the lungs and spleens of vaccinated animals. The vaccine induced high frequencies of IFNγ-secreting CD4+ T cells, as well as Th17 cells and lung-resident memory T cells (CXCR3+KLRG1−) that are known to be important for protective immunity against M. tb. When challenged with virulent M. tb, the NanoSTING-H1-vaccinated mice had significantly lower bacterial burdens in the spleens, livers and lungs than the unvaccinated controls. The protection provided by the intranasal NanoSTING-H1 vaccine was comparable to that of the subcutaneous Bacille Calmette-Guérin (BCG) vaccine, the only licensed TB vaccine. The vaccinated animals also exhibited less severe lung pathology and weight loss upon M. tb challenge.[27] This study demonstrated that the NanoSTING-adjuvanted intranasal H1 antigen vaccine can elicit potent cellular and humoral immune responses that protect against M. tb infection in a mouse model. The results suggest that the NanoSTING-H1 vaccine warrants further investigation as a next-generation TB vaccine candidate.

mRNA and Nucleic Acid Vaccines. RNA-based vaccines are an innovative strategy against pathogens with high mutation rates because mRNA vaccines can be easily designed by using new sequences encoding protective antigens and rapidly manufactured at scale. Recent research has developed and evaluated two mRNA vaccine candidates targeting *Pseudomonas aeruginosa* (PA): one encoding the type III secretion system protein PcrV (mRNA-PcrV-LNP) and another expressing a fusion of the outer membrane proteins OprF and OprI (mRNA-OprF-I-LNP).[28] Interestingly, both mRNA vaccines stimulated robust cellular and humoral immune responses in the immunized mice, with a balanced Th1/Th2 profile or a slight Th1 bias. The mRNA-PcrV-LNP vaccine induced significantly higher antibody titers and antigen-specific T cell responses than the mRNA-OprF-I-LNP vaccine. Vaccination using protein or mRNA vaccines provided broad protection against diverse PA strains in systemic and burn wound infection mouse models, outperforming the corresponding protein vaccines. Combining

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mRNA-OprF-I-LNP with mRNA-PcrV-LNP demonstrated the best survival and reduced bacterial burden in the organs compared with that of individual mRNA or protein vaccines.

Comparative studies of tuberculosis vaccination strategies revealed that replicating RNA (repRNA) and protein-adjuvant platforms differentially stimulate CD8+ and CD4+ T cell responses against distinct epitopes of the fusion antigen ID91, which incorporates four *M. tuberculosis* antigens.[29] In mouse challenge studies, heterologous prime-boost regimens combining the repRNA and protein-adjuvant platforms showed moderate additive effects in reducing the bacterial burden in the lungs compared with homologous regimens. The repRNA platform induced robust mucosal immunity and systemic antibody responses, as well as enhanced polyfunctional CD4+ and CD8+ T cell responses postchallenge, suggesting its potential to elicit a broader immune response against M. tb. The superior performance of mRNA vaccines compared with protein vaccines highlights the potential of this platform for developing effective prophylactic measures against antibiotic-resistant bacterial infections

Expanding the scope, a multivalent mRNA-LNP vaccine platform was developed against *Clostridioides difficile*, incorporating three key antigens: toxin A (TcdA), toxin B (TcdB), and the surface-layer protein SlpA.[30] Vaccination with the multivalent mRNA-LNP vaccine elicited robust antibody responses against all three antigens in mice, nonhuman primates, and humans. The vaccine-induced functional antibodies neutralized the cytotoxic effects of TcdB and TcdA *in vitro*. In mouse models, the multivalent vaccine provided complete protection against the lethal *C. difficile* challenge, whereas monovalent vaccines targeting individual antigens were less effective. Vaccination also reduced the gut bacterial burden, inflammation, and disease severity in a hamster model of the *C. difficile* infection. The protective efficacy of the mRNA-LNP vaccine was maintained even against a panel of diverse *C. difficile* strains, suggesting broad-spectrum coverage. The vaccine exhibited excellent tolerability in a phase 1 clinical trial involving healthy adults, and no serious adverse events were documented.

In a similar approach, an innovative multiepitope mRNA vaccine platform was developed for *Acinetobacter baumannii*, incorporating 10 highly conserved antigenic proteins screened for optimal CTL, HTL, and LBL epitopes using rigorous immunological and physicochemical criteria.[31] Three versions of the multiepitope mRNA vaccine (ABV1, ABV2, and ABV3) were designed, incorporating the selected epitopes along with different adjuvants (β-defensin 3, RS09, and CTB). These vaccine constructs exhibited stable interactions with key immune receptors (HLA-DRB101:01, HLA-A02:01, and TLR4) and showed promise in generating protective immunity against *A. baumannii* infection. Codon optimization and *in silico* insertion of the mRNA vaccine constructs into the pET28a(+) vector further supported their feasibility for large-scale production.

Recently, a novel DNA vaccine against *Helicobacter pylori* infection in mice was developed.[32] The Vaxign tool was used to identify the outer membrane protein LeoA as a potential *H. pylori* vaccine candidate. This DNA vaccine encoding the LeoA antigen was constructed and encapsulated within chitosan nanoparticles (Chitosan-LeoA-DNA nanoparticles), which had a spherical morphology, small size (150−250 nm), and positive surface charge with a high encapsulation efficiency (73.35%). Compared with the nonencapsulated DNA vaccine, the Chitosan-LeoA-DNA nanovaccine elicited higher levels of TNF-α and LeoA-specific IgG antibodies in vaccinated mice. Mice immunized with the nanovaccine showed 87.5% protection against *H. pylori* challenge, with reduced stomach inflammation and bacterial burden. The nanovaccine induced a shift from a Th1- to Th2-dominant immune response, mimicking the immune profile observed in *H. pylori*-infected individuals. *In vitro*, activated CD3+ T cells from nanovaccine-immunized mice inhibited the growth of human gastric cancer cells.[32] This study demonstrated that the Chitosan-LeoA-DNA nanoparticle-based vaccine effectively enhanced the immunogenicity and protective efficacy of the LeoA-DNA vaccine against *H. pylori* infection in a mouse model.

Vaccine-based Immunotherapy against Bacterial Resistance Mechanisms. Efflux Pump Outer Membrane Proteins (OMPs) as Vaccine Candidates. Efflux pumps are transmembrane proteins that actively extrude antibiotics and other harmful substances from bacterial cells, diminishing their intracellular levels and conferring resistance. These pumps contribute to both intrinsic and acquired resistance in bacteria. Gram-negative bacteria employ efflux pumps as a crucial mechanism for antimicrobial resistance. The upregulation of these efflux pumps has been detected in MDR strains,[33] rendering them appealing candidates for vaccine research and development. One approach involves developing vaccines that elicit an immune response to the efflux pump proteins. Silva and colleagues[34] showed that the *E. coli* TolC efflux pump protein is immunogenic and induces the production of protective antibodies. Inoculating mice with TolC resulted in improved survival rates following *E. coli* infection, indicating that using vaccines to target efflux pumps is a feasible strategy. This approach stimulates an immune response that inhibits efflux pump activity, restores antibiotic susceptibility, and offers several advantages, including the potential to target multiple MDR strains with a single vaccine.

Targeting Biofilms with Vaccines. Bacterial biofilms are complex assemblies of microorganisms surrounded by a self-generated matrix of extracellular polymeric substances (EPS). These biofilms display heightened resistance to antibiotics and immune defenses, posing challenges for eradication. Biofilm formation is a significant factor in chronic and medical device-related infections. Vaccination targeting SesC, a surface-exposed protein of *Staphylococcus epidermidis*, was shown to significantly reduce biofilm formation.[35] Antibodies against SesC inhibited colonization *in vitro* and in a mouse jugular vein catheter model. Active immunization with recombinant truncated SesC also inhibited foreign body infection in rats. Additionally, the surface protein G (SasG) of *Staphylococcus aureus* has been identified as an immunodominant antigen and a promising target for novel antibiofilm therapeutics.[36] Antibodies specifically aimed at purified recombinant SasG successfully impeded the biofilm formation. Furthermore, vaccine-generated antibodies targeting PilA, the major type IV pili subunit in nontypeable *Haemophilus influenzae* (NTHI), were shown to disrupt *Moraxella catarrhalis* in dual-species biofilms, enhancing bacterial susceptibility to antibiotics.[37]

Current Limitations in ESKAPE Vaccine Development. One of the most significant hurdles in bacterial vaccine development is the extensive antigenic variation and strain diversity of many bacterial species. Bacteria can evolve rapidly, altering their surface structures and virulence factors and rendering existing vaccines ineffective. For example, *Haemophilus influenzae* is divided into typeable and nontypeable strains, requiring different vaccine strategies.[39] The sequence diversity

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of transferrin-binding protein B (TbpB) in *Haemophilus influenzae* highlights this challenge, necessitating the development of vaccines that target conserved regions across multiple serotypes. The challenge is further exacerbated by the emergence of antibiotic-resistant strains, which can vary in their genetic characteristics, making it difficult to develop broadly protective vaccines.

Another major challenge is stimulating robust and long-lasting protective immunity against bacterial infections. Unlike viral infections, where neutralizing antibodies can often provide sterilizing immunity, bacterial infections often require a more complex immune response involving both humoral and cell-mediated immunity. For instance, facultative bacteria, such as *Salmonella enterica* and *Mycobacterium tuberculosis*, require the activation of CD8+ T cells and CD4+ T cells to clear the infection effectively.[40] Traditional vaccines that primarily induce antibody responses may not be sufficient to protect against these pathogens. The limited understanding of the components that define protective immunity against many bacterial infections hinders the development of these vaccines.

Demonstrating clinical efficacy in antibacterial vaccine trials can be particularly challenging, especially for vaccines targeting hospital-acquired infections. These trials often involve subjects with pre-existing immunity, temporary or chronic immunosuppression, and an unspecified microbiome status. This complexity makes it challenging to accurately assess the vaccine's true efficacy. Unpredictable rates of infection and evolving epidemiological conditions further complicate the trial design. Reevaluating research designs and expectations is necessary, with an emphasis on selecting the optimal immunological mechanism of action and timing for vaccination. In order to distinguish between populations that benefit from vaccination and those for whom vaccines may not be useful, it is crucial to enhance the characterization of patient subgroups within the trial population. This can be achieved by identifying immune and microbiological biomarkers. Genetic analysis and bioinformatic approaches could assist in the effective definition of these biomarkers effectively.

Effective vaccine delivery and formulation are critical for inducing strong and durable immune responses. Traditional vaccine delivery methods, such as intramuscular injection, may not be optimal for all bacterial infections, particularly those that primarily affect the mucosal surfaces. Mucosal vaccines, delivered through the nasal or oral route, can induce local and systemic immunity, providing a more effective barrier against infection. However, developing stable and effective mucosal vaccine formulations can be challenging. Nonetheless, novel delivery systems, such as nanoparticles and outer membrane vesicles (OMVs), offer promising avenues for improving vaccine delivery and enhancing immune responses.

Economic and logistical constraints also limit the development and deployment of bacterial vaccines, particularly in low- and middle-income countries. Developing, manufacturing, and distributing vaccines can be prohibitive, especially for diseases that primarily affect these regions. Ensuring vaccine accessibility and affordability is crucial to achieving global health equity. This requires innovative financing mechanisms, technology transfer, and local production capacity. Public−private partnerships and international collaborations are essential to overcoming these economic and logistical barriers.

Impact of Immunosuppression on Vaccine Efficacy. Immunocompromised individuals face unique challenges in achieving optimal vaccine protection due to limited clinical trial data and reduced immune responsiveness. Many vaccine studies exclude or inadequately represent this population, leading to gaps in understanding the safety and efficacy of immunization for those with weakened immune systems. As a result, these patients often exhibit diminished antibody production and cellular immune responses, leaving them susceptible to infections that vaccines typically prevent. To address this, researchers have explored alternative strategies, such as higher antigen doses, adjuvants, and novel delivery methods, to enhance vaccine effectiveness in this vulnerable group. Although inactivated vaccines are generally safe for immunocompromised patients, their protective benefits may be diminished. This is because the vaccines contain nonreplicating pathogens and pose no risk of causing active infection, making them a preferred option for this population. However, the weakened immune response in these individuals can lead to lower antibody titers and shorter-lasting immunity. Clinicians must therefore carefully evaluate the risks and benefits of vaccination on an individual basis, considering factors such as the patient's degree of immunosuppression and exposure risk to preventable diseases. Live attenuated vaccines, in contrast, require cautious administration due to the potential risk of vaccine-derived infection. These vaccines contain weakened but replication-competent pathogens, which can pose serious risks to individuals with a significant immune suppression. The decision to administer a live vaccine depends on the patient's specific immune status, with those undergoing intense immunosuppressive therapy at the highest risk. In select cases, the benefits of vaccination may outweigh the risks, but close monitoring and expert clinical judgment are essential to minimize adverse outcomes. Future research should focus on developing safer and more effective immunization strategies tailored to immunocompromised populations.

Future Directions in ESKAPE Pathogen Vaccine Development. Epitope-based vaccines, which target the vital regions of antigen molecules that initiate specific immune responses, represent a promising next-generation strategy. Through the rational combination of the dominant epitopes, these vaccines have the potential to elicit a more efficient and specific immune response compared with that of traditional vaccines. This targeted approach minimizes adverse reactions, improves efficacy, and optimizes protection against bacterial infections. However, epitope-based vaccines face challenges, such as epitope escape and low immunogenicity. To overcome these challenges, researchers are exploring strategies to enhance epitope presentation, improve vaccine stability, and incorporate adjuvants that stimulate strong T cell responses. For instance, incorporating adjuvants like human β-defensin 3 (HBD3) into recombinant proteins has been shown to enhance immunogenicity without compromising stability, as demonstrated in vaccines for *Acinetobacter baumannii*.[41]

Conjugate vaccines, which link weaker antigens such as cell wall glycans to carrier immunogenic proteins, have demonstrated significant efficacy against various bacterial infections. These vaccines leverage the immunogenic properties of carrier proteins to enhance the immune response to polysaccharide antigens, which are otherwise poorly immunogenic. The success of conjugate vaccines is evident in their application against diseases caused by pathogens such as *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* type b. The development of these vaccines has been marked by innovations in carrier proteins and conjugation methods, which have improved their effectiveness and broadened their

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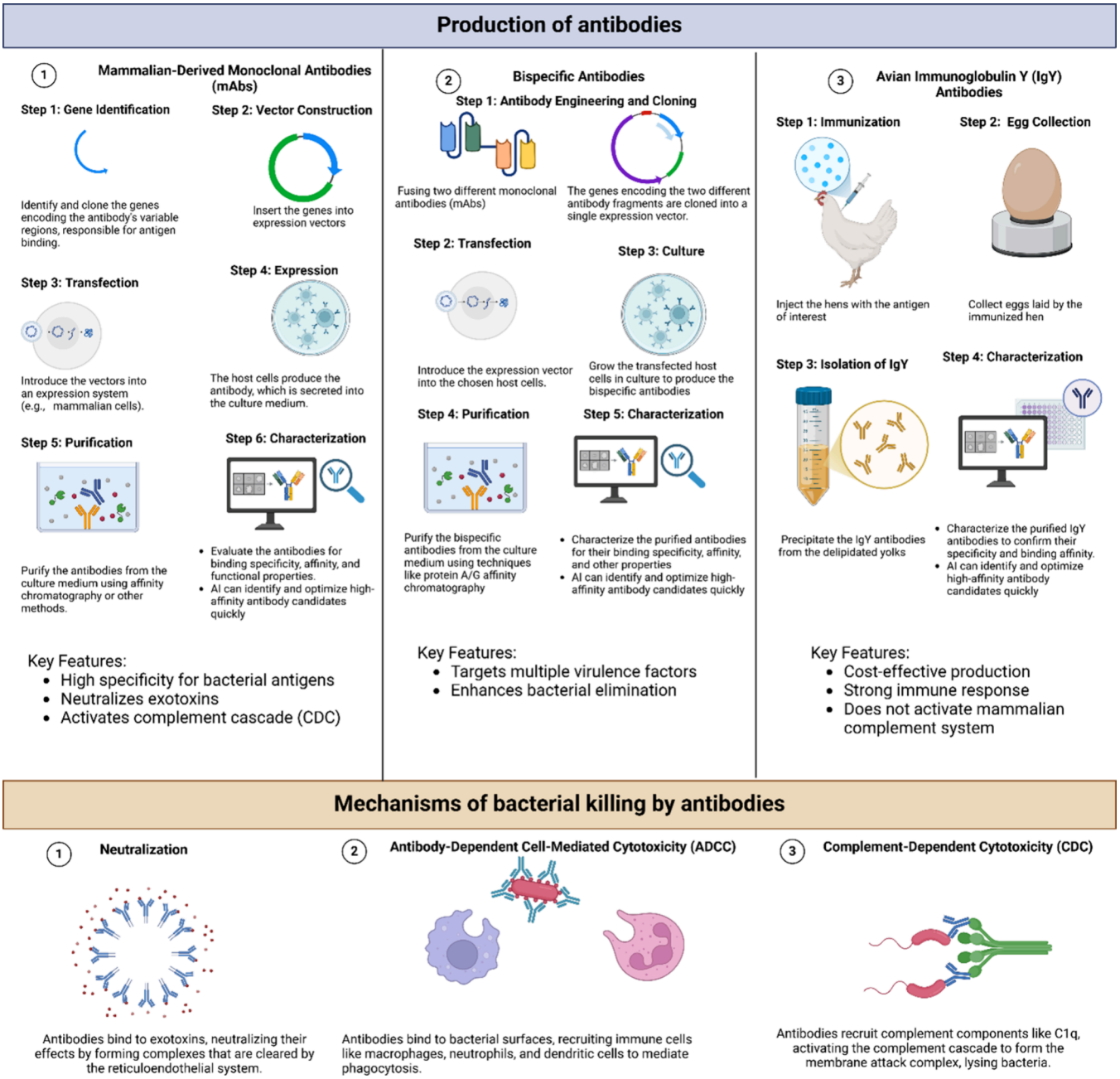

Figure 3. Illustration of the production and diversity of antibodies used in antibacterial immunotherapy. The upper panel of the figure shows the production of monoclonal antibodies (mAbs) (1), which involves generating identical copies of a single antibody molecule. This process ensures high specificity and affinity for the target antigen, which is essential for neutralizing bacterial toxins, activating complement systems, and mediating immune responses. mAbs have been widely studied for their therapeutic applications in combating bacterial infections. The production of bispecific antibodies (2) involves engineering antibodies that bind to two distinct bacterial targets simultaneously. This dual-targeting approach enhances therapeutic efficacy against drug-resistant bacterial strains. The production of Avian immunoglobulin Y (IgY) antibodies (3) involves immunizing birds with whole bacterial cells or purified bacterial antigens, followed by the extraction and purification of IgY antibodies from egg yolks. These antibodies offer several advantages, such as cost-effective production and strong immune responses, making them a promising alternative for antibacterial therapies. The bottom panel illustrates the mechanism of antibody killing. They can bind and neutralize bacterial toxins and virulence factors (1). They can also target bacterial surface antigens. Subsequently, effector immune cells, such as macrophages and neutrophils, recognize and bind to the antibody−antigen complexes on bacterial surfaces (2). This binding activates the complement system, leading to bacterial cell lysis and death (3).

applicability. Polysaccharides from bacterial cell surfaces are highly conserved and serve as excellent immunological targets. Advances in synthetic oligosaccharides and bioconjugation have enhanced the development of these antigens. Synthetic oligosaccharides facilitate the production of glycoconjugate vaccines and serve as tools for in-depth mechanistic investigations into vaccine immunology. Future research should focus on expanding conjugate vaccines to target a wider range of bacterial pathogens and developing more efficient and cost-effective conjugation methods.

Nanoparticle-based vaccines offer several advantages for ESKAPE pathogen vaccine development, including improved antigen delivery, enhanced immune responses, and the ability to target specific immune cells. Using nanoparticles allows for

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incorporating bacterial components, such as outer membrane vesicles, to enhance immunogenicity and protect against severe infections such as pneumonia and sepsis. For instance, outer membrane vesicle-coated nanoparticles have been developed to protect against *Acinetobacter baumannii*.[42] The vaccine combines immunogenic outer membrane vesicles (OMVs) derived from *A. baumannii* with stabilizing gold nanoparticle (AuNP) cores to create an *A. baumannii* nanoparticle vaccine (Ab-NP). Ab-NP vaccination induced robust *A. baumannii*-specific IgG antibody responses in both rabbits and mice, and the antisera effectively promoted the opsonophagocytic killing of *A. baumannii* by human neutrophils. Passive immunization with Ab-NP immune serum protected mice against lethal *A. baumannii* sepsis. Active Ab-NP vaccination also protected mice against both lethal sepsis and pneumonia caused by a highly virulent *A. baumannii* strain. The nanoparticle platform provided improved consistency and stability compared with OMVs alone, addressing the manufacturing challenges associated with traditional OMV-based vaccines. Nanoparticle-based vaccines are particularly promising for targeting intracellular bacterial pathogens as they can be engineered to enhance both humoral and cell-mediated immune responses. These vaccines can improve survival outcomes in *in vivo* models by enhancing antigen presentation and inducing robust immune responses. While nanoparticle-based vaccines show great promise, challenges remain in their development and application. Issues such as the complexity of production, possible adverse reactions, and the requirement for thorough clinical trials need to be tackled to maximize their benefits.

Mucosal vaccines, administered through nasal or oral routes, present a promising strategy for preventing bacterial infections by inducing both mucosal and systemic immunity. These vaccines target the mucosal surfaces, the primary entry points for many pathogens, offering a first line of defense. Intranasal vaccines, for instance, have shown the ability to induce high levels of IgA and IgG antibodies, which are crucial for neutralizing pathogens at mucosal surfaces and blocking their entry through the nasal passages, making them effective against respiratory infections. This approach facilitates a robust immune response and improves patient compliance due to its non-invasive nature. Developing mucosal vaccines involves innovative delivery systems and adjuvants to enhance their efficacy and stability. Nanoparticle-based delivery systems, such as PEGylated lipids and carbonate apatite nanoparticles, have been developed to protect antigens from degradation and facilitate targeted delivery to immune cells, thereby ensuring effective mucosal vaccination. Future research should focus on developing more stable and effective mucosal vaccine formulations and identifying optimal delivery routes and adjuvants.

■ ANTIBODY-BASED IMMUNOTHERAPY

Antibodies play a crucial role in antibacterial immunotherapy, and various types of antibodies are being explored for their potential therapeutic applications. The most widely studied are mammalian-derived monoclonal antibodies (mAbs). The production of mAbs involves generating identical copies of a single type of antibody molecule. In addition to conventional monoclonal antibodies, researchers have developed bispecific antibodies that are engineered to bind to two distinct bacterial targets simultaneously. This is typically achieved through genetic engineering techniques, allowing the bispecific antibody to engage multiple virulence factors or surface proteins on the bacterial pathogen. An alternative approach is using avian immunoglobulin Y (IgY) antibodies, which are derived from the egg yolks of immunized chickens or other egg-laying birds. The production of IgY antibodies involves immunizing the birds with whole bacterial cells or purified bacterial antigens, after which IgY antibodies are extracted from egg yolks (Figure 3). Machine learning (ML) or artificial intelligence (AI) is revolutionizing antibody production by enhancing efficiency and accuracy. AI algorithms can predict the structure and binding affinity of antibodies, reducing the need for extensive experimental screening and increasing the likelihood of discovering effective antibodies (Figure 3).

The diverse antibody types, each with unique strengths and mechanisms of action, provide a versatile arsenal against persistent and drug-resistant ESKAPE bacterial infections. Generally, these antibodies exert their actions by recognizing specific antigens and mediating their effects through various mechanisms, including the activation or inhibition of cell surface receptors, as well as antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), as shown in Figure 3. When antibodies function through neutralization mechanisms, they typically target exotoxins. By binding to toxin receptors, these antibodies form complexes cleared by the reticuloendothelial system. Furthermore, antibodies that adhere to the surfaces of bacteria can improve the binding and recruitment of soluble complement components, such as C1q, which activates the complement cascade, forms the membrane assault complex, and ultimately eliminates bacteria. Antibodies can also induce the phagocytosis of bacteria by macrophages, neutrophils, and dendritic cells by binding to bacterial surface antigens (Figure 3).

Research, Case Studies, Preclinical and Clinical Trials. *Mammalian Monoclonal Antibodies.* Researchers have investigated the use of mAbs against different targets, including α-toxin, surface protein adhesions, biofilms, the immunoglobulin-binding protein SpA, teichoic acid, and capsular polysaccharides (Table 2). As of December 2019, the U.S. FDA had approved 79 therapeutic monoclonal antibodies, with comprehensive listings available in published literature.[43] The introduction of humanized antibodies has revolutionized the field of monoclonal antibody therapy. This innovative antibody engineering strategy involved replacing the murine Fc and Fv regions with human germline amino acid sequences, significantly reducing immunogenicity.[44]

Klebsiella pneumoniae is a significant nosocomial pathogen known for its extensive drug resistance. The development of MDR strains of *K. pneumoniae* poses a significant challenge for healthcare providers. There is an increasing incidence of *K. pneumoniae* lipopolysaccharide O2 serotype strains in several drug resistance groups. Interestingly, research has successfully identified human monoclonal antibodies that target O-antigens with remarkable efficacy. In animal models of infection, these antibodies provide significant protection against densely encapsulated strains. Among these antibodies, of particular note are uncommon and distinct anti-O2 antibodies, which, in combination with the often-prescribed antibiotic Meropenem, work in concert to protect against drug-resistant *K. pneumoniae*.[45]

A promising humanized mAb candidate (A1102) has been developed to target the KPC-producing MDR *Klebsiella pneumoniae* strains.[46] Preclinical investigations have validated the efficacy of A1102 in safeguarding against *K. pneumoniae* infections. Passive administration of A1102 prior to a lethal

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challenge with either ST258 whole bacteria or ST258-derived lipopolysaccharide (LPS) has been shown to prolong the survival of endotoxin-sensitized mice and protect rabbits exposed to a lethal ST258 challenge. *In vitro* analyses revealed that the biological activities of A1102 include complement- and Fc-independent neutralization of LPS via boosting human serum bactericidal activity and promoting the complement-dependent phagocytosis of ST258 by macrophages.

Moreover, novel broadly reactive IgG monoclonal antibodies have been developed against carbapenem-resistant *Klebsiella pneumoniae* ST258 strains.[47] Immunizing mice with a blend of CR *K. pneumoniae* capsular polysaccharide (CPS) linked to an anthrax protective antigen resulted in the production of IgG mAbs 17H12 and 8F12, which exhibited affinity for clade 2 ST258 CR *K. pneumoniae* CPS. These mAbs facilitated various extracellular and intracellular killing mechanisms against clade 2 CR *K. pneumoniae*, including biofilm inhibition, complement activation, neutrophil extracellular trap formation, and opsonophagocytic and intracellular eradication. In a murine intratracheal infection model, pre-opsonization of clade 2 CR *K. pneumoniae* with 17H12 or 8F12 reduced bacterial dissemination to the lungs, liver, and spleen compared with the control groups.

Focusing on a different target, researchers have discovered and characterized mAbs that target the type 3 fimbrial (T3F) protein MrkA in *K. pneumoniae*.[48] A target-independent phage display approach was used to screen live *K. pneumoniae* strains, including wild-type and capsular/LPS-deficient mutants. Interestingly, carbohydrate-targeting mAbs were rare, and most of these antibodies targeted proteinaceous epitopes. Several highly prevalent mAbs bound to the T3F subunit MrkA, a known virulence factor. The mAbs directed against MrkA showed extensive cross-reactivity by binding to various *K. pneumoniae* clinical isolates, encompassing various O-serotypes. In opsonophagocytic killing (OPK) assays, MrkA-targeting mAbs promoted the killing of the acapsular *K. pneumoniae* strain, but this effect diminished over time. High-content imaging revealed heterogeneity in the surface expression of T3F within the bacterial population, with some bacteria completely lacking MrkA expression. Heterogeneity in MrkA expression may explain the modest therapeutic efficacy of anti-MrkA mAbs reported in previous *in vivo* experiments. The findings highlight the importance of considering bacterial heterogeneity in the development of antibody-based therapeutics. The effectiveness of mAbs depends on several factors, including the target antigen, the antibody isotype, and the host immune response. Some studies suggest that IgG3 mAbs may offer superior protection against *K. pneumoniae* compared with IgG1 mAbs. However, further research is needed to fully understand the optimal characteristics of mAbs for treating bacterial infections.

The treatment of MRSA infections presents a significant challenge because the bacterium has developed resistance to numerous antibiotics, including methicillin, oxacillin, and other β-lactam antibiotics. Consequently, alternative therapeutic approaches, such as immunotherapies involving mAbs, are actively under investigation to combat MRSA infections. For example, Boechat and colleagues[49] developed a recombinant Fab fragment derived from a mAb targeting the penicillin-binding protein 2a (PBP2a) of MRSA. The anti-PBP2a Fab exhibited strong binding affinity to both the native and recombinant forms of PBP2a. *In vivo* exposure in mice revealed that anti-PBP2a Fab had a plasma half-life of 6−8 h, shorter than the reported half-life of the F(ab′)2 fragment. Biodistribution analysis showed that Fab fragments were present in the spleen, kidneys, serum, and lungs, indicating a broad tissue distribution. The shorter half-life of Fab fragments compared with those of larger antibody formats may offer advantages in certain infection scenarios by improving tissue penetration. These findings highlight the potential of anti-PBP2a Fab as a versatile therapeutic tool for combating MRSA infections.

Researchers have also investigated using mAbs against *P. aeruginosa*, a significant opportunistic pathogen. For instance, ammonium metavanadate was utilized during *P. aeruginosa* cultivation to induce stress responses and boost polysaccharide production.[50] Mice were immunized with *P. aeruginosa* cultured in the presence of ammonium metavanadate, leading to the generation of two IgG2b mAbs, WVDC-0496 and WVDC-0357, which specifically targeted the O-antigen lipopolysaccharide of *P. aeruginosa*. These mAbs directly promoted clumping and decreased the bacterial viability in the functional tests. Prophylactic doses as low as 15 mg/kg WVDC-0496 and WVDC-0357 resulted in 100% survival in a fatal sepsis model. Both mAbs significantly decreased the bacterial load and inflammatory cytokine levels in the sepsis and pneumonia models. An alternative approach used conserved bacterial flagellin peptides to generate broadly reactive IgG2b monoclonal antibody WVDC-2109, specifically targeting *P. aeruginosa*.[51] The *in vitro* evaluation of WVDC-2109 revealed complement-mediated bactericidal effects and enhanced the opsonophagocytosis of *P. aeruginosa*. Prophylactic administration of WVDC-2109 significantly improved survival and outcomes in a lethal sepsis model and a sublethal murine pneumonia model of *P. aeruginosa* infection, reducing bacterial burden and inflammation.

A recent study introduced an innovative antibody−drug conjugate (ADC) platform demonstrating promising therapeutic potential.[52] ADCs combine the precise targeting capability of a mAb with the potent antimicrobial properties of an antimicrobial peptide. To specifically target *P. aeruginosa*, the researchers created an ADC by integrating an antimicrobial peptide into the VL and/or VH chains of a mAb known as VSX. This antibody was designed to specifically recognize the core of *P. aeruginosa* lipopolysaccharides. The ADC displayed low toxicity toward mammalian cells, successfully eliminated different strains of *P. aeruginosa*, and offered therapeutic defense against *P. aeruginosa* lung infection in mice.

Moreover, a compelling new strategy that simultaneously targets two virulence factors has emerged. Researchers developed a bispecific antibody to simultaneously bind the Psl exopolysaccharide and the PcrV component of the type III secretion system, both of which are critical for the virulence of *P. aeruginosa*.[53] *In vitro* experiments demonstrated that the bispecific antibody significantly improved neutrophil phagocytosis and the killing of *P. aeruginosa* compared with the control antibody. In a mouse model of *P. aeruginosa* lung infection, administering the bispecific antibody enhanced bacterial clearance and reduced inflammation. Furthermore, in a clinical trial involving patients suffering from bronchiectasis and chronic *P. aeruginosa* infection, the bispecific antibody increased neutrophil-mediated bacterial killing *ex vivo* compared with the baseline. The ability of this bispecific antibody to enhance neutrophil function by targeting two essential virulence factors suggests that it could serve as a promising immunotherapeutic approach for *P. aeruginosa* infections in patients with bronchiectasis. Overall, this study highlights the potential of rationally designed bispecific antibodies to boost innate immune

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responses and improve clinical outcomes in patients with chronic *P. aeruginosa* lung infections.

A recent study developed broadly protective monoclonal antibodies (mAbs 8E6 and 1B5) against MDR *Acinetobacter baumannii* by sequentially immunizing mice with sublethal doses of three pan-drug-resistant strains (ST-208, ST-195, and ST-229).[54] Both mAbs were shown to effectively protect against respiratory infections within 4−24 h by stimulating the release of innate immune factors and inflammatory cytokines, thereby reducing the duration of illness in mice. By targeting the ATP synthase antigens, mAb 8E6 and mAb 1B5 significantly enhanced the opsonization process of phagocytosis, leading to protective effects in mice. Interestingly, research uncovered striking structural similarities between the *C. albicans* Hyr1 protein and *A. baumannii* cell surface antigens, suggesting potential evolutionary convergence.[55] The anti-Hyr1 mAbs not only impede the damage to primary endothelial cells caused by *A. baumannii* but also protect mice against fatal pulmonary infections. This investigation underscores the potential of using Hyr1p mAbs as a cross-kingdom immunotherapeutic strategy against MDR Gram-negative bacteria. In addition to these advancements, researchers have developed an antibody that targets a component of the bacterial cell surface called pseudomaphyseic acid (Pse). The antibody Pse-MAB1 killed various strains of *A. baumannii* without host immune factors. This represents an exciting research direction for treating infections in patients with a compromised immune system. Furthermore, Pse-MAB1 was found to protect mice against *A. baumannii* infection.[56]

Antibiotic treatment for *Clostridioides difficile* infection (CDI) can disrupt the gut microbiota, leading to recurrent infections. Bezlotoxumab is a mAb that neutralizes toxin B, a major virulence factor of *C. difficile*. It has received FDA approval to prevent recurrent CDI in adults undergoing antibiotic treatment. Clinical trials have demonstrated that bezlotoxumab, when administered with antibiotics (vancomycin, fidaxomicin, or metronidazole), significantly decreases the risk of CDI recurrence compared with antibiotics alone.[57] In a phase 3 trial, bezlotoxumab significantly reduced the incidence of recurrent CDI, the necessity for faecal microbiota transplants, and the rate of CDI-associated readmissions within 30 days compared with placebo among participants with risk factors for recurrent CDI. The most significant reduction was seen in participants with ≥3 risk factors.[58]

Mammalian Polyclonal Antibodies. Unlike mAbs, polyclonal antibodies have been less explored. Recently, Seixas and colleagues[59] investigated the inhibitory properties of an anti-BCAL2645 goat polyclonal antibody. They observed that this antibody effectively counteracted the formation of biofilms by *P. aeruginosa* and impeded its interaction with the human bronchial epithelial cell line CFBE41o−. Positively, the results demonstrated a noteworthy decrease in biofilm development and interference of the antibody with the interaction of *P. aeruginosa* with CFBE41o−. Remarkably, these bacterial strains showed reduced larval mortality when they were treated with the anti-BCAL2645 antibody before infection. Passive immunotherapy is a promising alternative to traditional antibiotics, but the high cost of mammalian-sourced antibodies hinders its large-scale production.

Avian Immunoglobulin Y (IgY) Antibodies. Compared with mammalian IgG, IgY antibodies from egg-laying hens provide a high-yield and affordable alternative. Recent studies have demonstrated that anti-DEC IgY effectively combats diarrheagenic *Escherichia coli* through multiple mechanisms. *In vitro* growth inhibition assays revealed significant suppression at a concentration of 25 mg/mL, whereas *in vivo* testing showed that a concentration of 12 mg/mL reduced intestinal colonization and infection severity in mouse models.[60] These findings highlight IgY immunotherapy's potential against antibiotic-resistant bacterial infections.

Focusing on *Streptococcus pneumoniae*, researchers successfully developed and characterized chicken-derived antibodies targeting the recombinant Eno1 protein (spEno1), which interacts with human plasminogen, a crucial extracellular matrix component.[61] Chickens were immunized with purified spEno1 protein to generate polyclonal IgY antibodies that exhibited strong binding activity to spEno1. In addition, two scFv antibody libraries were constructed using phage display technology, identifying 10 unique scFv clones through biobanking. These scFv antibodies recognized the recombinant spEno1 and endogenous Eno1 proteins expressed by *S. pneumoniae*. Several scFv antibodies, including spEnS10, spEnS9, and spEnS8, effectively inhibited the interaction between the plasminogen and spEno1. The scFv antibodies targeting spEno1 show promise as diagnostic and therapeutic agents for *S. pneumoniae* infections. Further optimization, such as antibody affinity maturation and Fc fusion engineering, can enhance their binding affinity and functional properties, paving the way for their clinical application.

Expanding the scope to *Pseudomonas aeruginosa* (PA), researchers evaluated polyclonal avian IgY antibodies raised against inactivated PAO1 whole cells, demonstrating protective efficacy in murine models of both burn wound infections and acute pneumonia. The anti-PAO1 IgY exhibited significantly higher titers and cross-reactivity against the standard PA strains (PAO1 and PAK) than the control IgY (C-IgY). Immune responses induced by anti-PAO1 IgY remained robust for up to 14 weeks after the final injection. Moreover, the anti-PAO1 IgY successfully hindered the growth, movement, biofilm formation, and internalization of various PA strains in a dose-dependent manner. It also bolstered the opsonophagocytic killing of PA by polymorphonuclear leukocytes. In passive immunotherapy trials, anti-PAO1 IgY offered complete protection against lethal PA infections in both acute pneumonia and burn wound models. Additionally, it significantly decreased the bacterial levels in the spleen, liver, and blood of the burned mice compared with the control mice. These results highlight the potential of this passive immunotherapy strategy in combating *Pseudomonas aeruginosa* infections.

Vibrio cholerae, a Gram-negative bacterium, causes cholera, a serious diarrheal illness. The cholera toxin produced by *V. cholerae* disrupts normal ion transport in the intestines, leading to significant fluid and electrolyte loss. A novel cholera vaccine approach utilized a recombinant OTC fusion protein (containing OmpW, TcpA, and CtxB) to elicit potent IgY responses in hens, targeting multiple pathogenic mechanisms simultaneously.[63] Anti-OTC IgY antibodies demonstrated strong immunoreactivity against the chimeric protein and its individual antigen components in the ELISA assays. In the cell-based assays, anti-OTC IgY at 250 μg/mL effectively neutralized the cytotoxic effects of cholera toxin (CT). Two oral doses of 100 μg of anti-OTC IgY provided 60% protection against lethal doses and 20% protection against 10-fold lethal doses of *V. cholerae* in infant mouse challenge experiments. This level of protection was superior to that provided by IgY antibodies against individual antigens and their mixtures. These findings highlight the

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potential of anti-OTC IgY as a promising passive immunotherapy strategy for cholera prevention and treatment.

Antibody Engineering and Delivery. Advances in screening and engineering methods have significantly expanded the therapeutic capabilities of the mAbs. Recent research has introduced an Fc-engineered antibody format called REW, featuring three key amino acid substitutions (Q311R/M428E/N434W) to enhance the therapeutic properties.[64] This new molecule offers multiple benefits, including an extended plasma half-life, improved distribution in mucosal tissues, and the ability to traverse respiratory epithelial barriers without needles. This characteristic has a commercially competitive advantage because it affects dosing and the frequency of administration and potentially improves patient compliance. Most importantly, the Fc-engineered variant enhanced *S. aureus* phagocytosis. These findings suggest that this versatile Fc technology has broad applicability in designing antibodies for long-acting prophylactic or therapeutic interventions.

Exploring mRNA-based approaches, researchers developed and evaluated an mRNA platform for preventing and treating *Staphylococcus aureus* infections through the neutralization of staphylococcal enterotoxin B (SEB).[65] The platform features an anti-SEB mRNA antibody, offering several advantages over traditional antibody therapies. The anti-SEB mRNA antibody maintained continuous secretion of the anti-SEB mAb at a dosage 10× lower than that needed for administering the purified protein. Additionally, it demonstrated enhanced pharmacokinetic characteristics compared with the purified anti-SEB mAb, effectively neutralizing SEB and eliminating *S. aureus* from the circulation. This study establishes a proof-of-concept for mRNA-based methods targeting SEB toxins, delivering robust protection and efficient treatment against *S. aureus* infections.

Moreover, the exploration of targeting immune evasion proteins, such as staphylococcal protein A (SpA), which is found in *S. aureus*, has been extensive. The effectiveness of antibodies is typically impaired in patients with SpA; however, scientists have discovered that SpA cannot bind to a specific subtype of human antibody called IgG3 because of a substitution in one of the nine Fc-contact residues in IgG3. The residue Arg435 in IgG3 causes steric hindrance to SpA when it binds to IgG3-Fc. This discovery led to the identification of the potent antibody 514G3, which has shown promising results in preventing *S. aureus* bacteraemia. Clinical studies have shown that this antibody can shorten hospitalization times for patients with MRSA bacteraemia.[66] Further investigations are planned for a phase II clinical study to explore the potential efficacy.

Recent research has validated adeno-associated virus (AAV) vectored immunoprophylaxis as an effective strategy for the sustained production of protective monoclonal antibodies (mAbs) against *Pseudomonas aeruginosa*.[67] AAV vectors expressing anti-PcrV mAb (AAV-αPcrV), anti-Psl mAb (AAV-αPsl), or the bispecific mAb MEDI3902 (AAV-MEDI3902) were generated and characterized. When administered intramuscularly, these AAV-mAb vectors provided significant protection against the lethal intranasal challenges of *P. aeruginosa* strains PA14 and PAO1 in mice. AAV-MEDI3902 and AAV-αPcrV provided the highest levels of protection, with 100% and 87.5% survival, respectively, against the lethal PAO1 challenge and 87.5% and 75% survival, respectively, against the lethal PA14 challenge. Compared with the individual mAb vectors, mice treated with AAV-MEDI3902 demonstrated a greater reduction in bacterial spread to the blood, spleen, lung, and liver despite having serum antibody concentrations ∼10-fold lower than those of AAV-αPcrV. This study demonstrated the potential of AAV-delivered monospecific and bispecific mAbs as effective prophylactic and therapeutic strategies against lethal *P. aeruginosa* pneumonia in a mouse model, laying the groundwork for the development of novel interventions against this important bacterial pathogen.

Antibody-based Immunotherapy Targeting Bacterial Resistance Mechanisms. *Targeting Resistance Enzymes with Antibodies.* Bacteria secrete enzymes that alter or break down antibiotics, making them ineffective. These enzymes, including β-lactamases, aminoglycoside-modifying enzymes, and chloramphenicol acetyltransferase, play a significant role in and are a major cause of antibiotic resistance. Targeting resistance enzymes with antibodies presents substantial advantages, including restoring antibiotic efficacy by inhibiting these enzymes, which can also allow for broad-spectrum activity by reversing resistance to multiple antibiotics. Additionally, this approach helps prevent the spread of resistance genes by reducing the selective pressure. Strategies for developing such antibodies include direct binding to the enzyme's active site to block substrate access, inducing conformational changes that disrupt enzyme function, and engineering antibodies to promote the degradation of resistance enzymes, thereby limiting their activity and availability. Recent advances have produced camelid-derived heavy-chain antibodies (VHHs/nanobodies) that specifically inhibit CMY-2 β-lactamase. Structural analysis of the cAbCMY-2(254)/CMY-2 complex revealed the epitope's proximity to the active site, CDR3 insertion into the catalytic pocket, and mixed inhibition (predominantly noncompetitive). These competitive-binding VHHs recognize overlapping epitopes, enabling both β-lactamase inhibition and the development of diagnostic ELISAs for detecting CMY-2 β-lactamase, which can be crucial for identifying resistant bacterial strains.[68] Developing these antibodies represents a novel approach to combating bacterial resistance by targeting the enzymes that degrade antibiotics.

Although developing antibodies against β-lactamase is a significant advancement, the complexity of β-lactamase enzymes and their diverse structural motifs pose ongoing challenges. The plasticity of the β-lactamase active site contributes to its wide resistance to existing inhibitors, necessitating continuous research and innovation in this field.

Targeting Bacterial Biofilms with Antibodies. Targeting biofilms with antibodies presents several advantages, including the disruption of the biofilm structure leading to bacterial dispersal, enhanced antibiotic penetration by improving susceptibility, and facilitating immune cell access for phagocytosis and bacterial killing. Strategies for antibody-mediated biofilm disruption involve targeting the extracellular matrix components like polysaccharides and proteins, binding to bacterial adhesins to impede attachment and biofilm initiation, and interfering with quorum sensing to disrupt bacterial communication and inhibit biofilm formation effectively. Several studies have demonstrated the potential of antibodies to disrupt bacterial biofilms. For instance, researchers have developed antibodies that bind to the surface proteins and extracellular polysaccharides of *S. aureus* biofilms, disrupting the biofilm structure and enhancing the antibiotic efficacy.[69] Antibodies targeting alginate, a major component of the *P. aeruginosa* biofilm matrix, disrupt biofilms and improve antibiotic susceptibility.[70] Extracellular DNA (eDNA) is a structural component of biofilms, and antibodies that bind to

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DNABII proteins, integral components of eDNA, can disrupt biofilm formation and stability.[71]

Limitations and Challenges of mAb Therapy. Although mAbs have shown promise in targeting specific bacterial virulence factors, toxins, and surface antigens (Table 2), their overall therapeutic efficacy is limited by several key challenges that have hindered their wider adoption for treating bacterial infections.

One major challenge is the significant heterogeneity observed in the bacterial populations. Many pathogens, such as *Klebsiella pneumoniae*, exhibit remarkable diversity in the expression of their target antigens. This can lead to a subset of bacteria evading recognition and killing by mAbs that target a single epitope. Furthermore, the sophisticated immune evasion mechanisms employed by bacteria, such as the production of immunoglobulin-binding proteins such as *Staphylococcus aureus* protein A (SpA) and the formation of polysaccharide capsules, can physically shield their surface antigens from antibody recognition and binding. This impairs the effectiveness of mAbs even when targeting specific virulence factors.

The rapid evolution of bacterial pathogens presents another significant challenge, as it can lead to the emergence of resistant strains capable of evading the effects of mAbs. This is particularly problematic for mAbs targeting a single epitope as mutations in that specific epitope can confer antibody resistance. For example, researchers have observed the development of resistance to anti-PBP2a mAbs in MRSA strains, where mutations in the PBP2a target can render the antibody ineffective[49] The large size of the full-length mAbs (approximately 150 kDa) can also limit their ability to penetrate certain tissues effectively and reach the site of infection, particularly in the case of deep-seated or hard-to-reach infections. This challenge is especially relevant for infections involving biofilms or intracellular pathogens, such as *Mycobacterium tuberculosis*, where the antibody may struggle to access the bacteria.

Another challenge is the potential for immunogenicity, as murine-derived mAbs can elicit an immune response in human recipients, resulting in the production of antidrug antibodies (ADAs) that can neutralize the therapeutic effect and increase the risk of adverse events.[72] Although the development of humanized and fully human mAbs has reduced this concern, the potential for immunogenicity remains a challenge, especially in immunocompromised patients or with repeated dosing.

The clinical use of mAb therapies is often hindered by logistical challenges, particularly in low-resource and underserved regions. As these treatments typically require intravenous infusion, patients may need hospitalization or frequent visits to specialized medical centers, creating accessibility issues for those in remote areas. Furthermore, the short half-life of mAbs necessitates repeated administration to sustain effective drug concentrations, increasing treatment costs and patient burden. Finally, mAb production remains constrained by complex manufacturing requirements and high costs, including specialized cell culture systems, extensive purification processes, and rigorous quality control, which limit accessibility in resource-limited settings.

Failures and Challenges Faced in mAbs Trials. The development and clinical evaluation of mAb therapies for bacterial infections face multiple obstacles, including target selection, understanding protective mechanisms, and regulatory complexities. Bacterial pathogenesis is highly intricate, and diverse patient populations make it difficult to design clinical trials that reliably assess the therapeutic efficacy and safety. Early attempts using polyclonal antilipid A antiserum failed to demonstrate significant protection in sepsis trials. Despite targeting multiple epitopes, polyclonal antibodies did not reduce mortality, suggesting that lipid A alone may be an insufficient target. This could be due to structural variations in the lipopolysaccharide (LPS) across bacterial species or the influence of additional virulence factors. Consequently, research has shifted toward mAbs, which provide greater specificity but still encounter substantial challenges. For instance, clinical trials investigating mAbs targeting the lipid A component of LPS, a key mediator of immune responses to Gram-negative bacteria, have yielded inconsistent results. Although lipid A is highly conserved and toxic, neutralizing it with mAbs did not significantly reduce sepsis mortality in large-scale studies. Several factors may explain this failure, including patient heterogeneity, the multifactorial nature of sepsis, and the limitations of targeting a single inflammatory pathway. Notably, some trials reported increased mortality in patients without Gram-negative bacteraemia who received antilipid A mAbs.[73] This raised concerns about potential off-target effects, such as immune system disruption and unintended toxicity, in uninfected individuals.

In addition, two mAbs, E5 (a murine IgM) and HA-1A (a human IgM), were developed as targeted antiendotoxin therapies to improve outcomes in Gram-negative infections. Both antibodies specifically bind to lipid A component of endotoxin (LPS), aiming to neutralize its toxic effects and block the excessive inflammatory response characteristic of sepsis. Despite strong preclinical evidence supporting its efficacy, E5 failed to demonstrate a significant survival benefit in clinical trials involving patients with Gram-negative infections who did not present with refractory shock.[74] This discrepancy between laboratory results and real-world clinical outcomes underscores difficulties in translating experimental success into therapeutic effectiveness. The lack of benefit in nonshock patients suggests that targeting lipid A alone may be insufficient in specific sepsis subgroups, possibly due to the complexity of the host immune response or variations in bacterial virulence factors.

Furthermore, bispecific antibody gremubamab (MEDI3902) targets two key *Pseudomonas aeruginosa* virulence factors: PcrV (mediating host cell cytotoxicity) and Psl (essential for colonization and tissue adherence). Preclinical evaluation in rabbit models of acute pneumonia demonstrated reduced bacterial burden, diminished tissue damage, and improved pulmonary function and survival. Subsequent phase 1b/2a clinical trials assessed MEDI3902's safety and efficacy in mechanically ventilated patients at high risk of *P. aeruginosa* pneumonia.[75] However, the trial failed to meet its primary end point, with MEDI3902 (1500 mg) showing no significant reduction in *P. aeruginosa* pneumonia incidence versus the placebo (22.4% vs 18.1%; relative risk reduction −23.7%). Posthoc analyses indicated potential efficacy in subgroups with lower baseline inflammation (procalcitonin/neutrophil levels). Despite being well-tolerated with a placebo-comparable safety profile, MEDI3902 did not prevent pneumonia in the overall cohort, suggesting the need for either dose optimization or patient stratification in future studies.

Collectively, these challenges emphasize the difficulties in developing effective mAb-based therapies for bacterial infections. To overcome these hurdles, future research must prioritize a more comprehensive understanding of bacterial pathogenesis, refined patient stratification, and improved trial methodologies.

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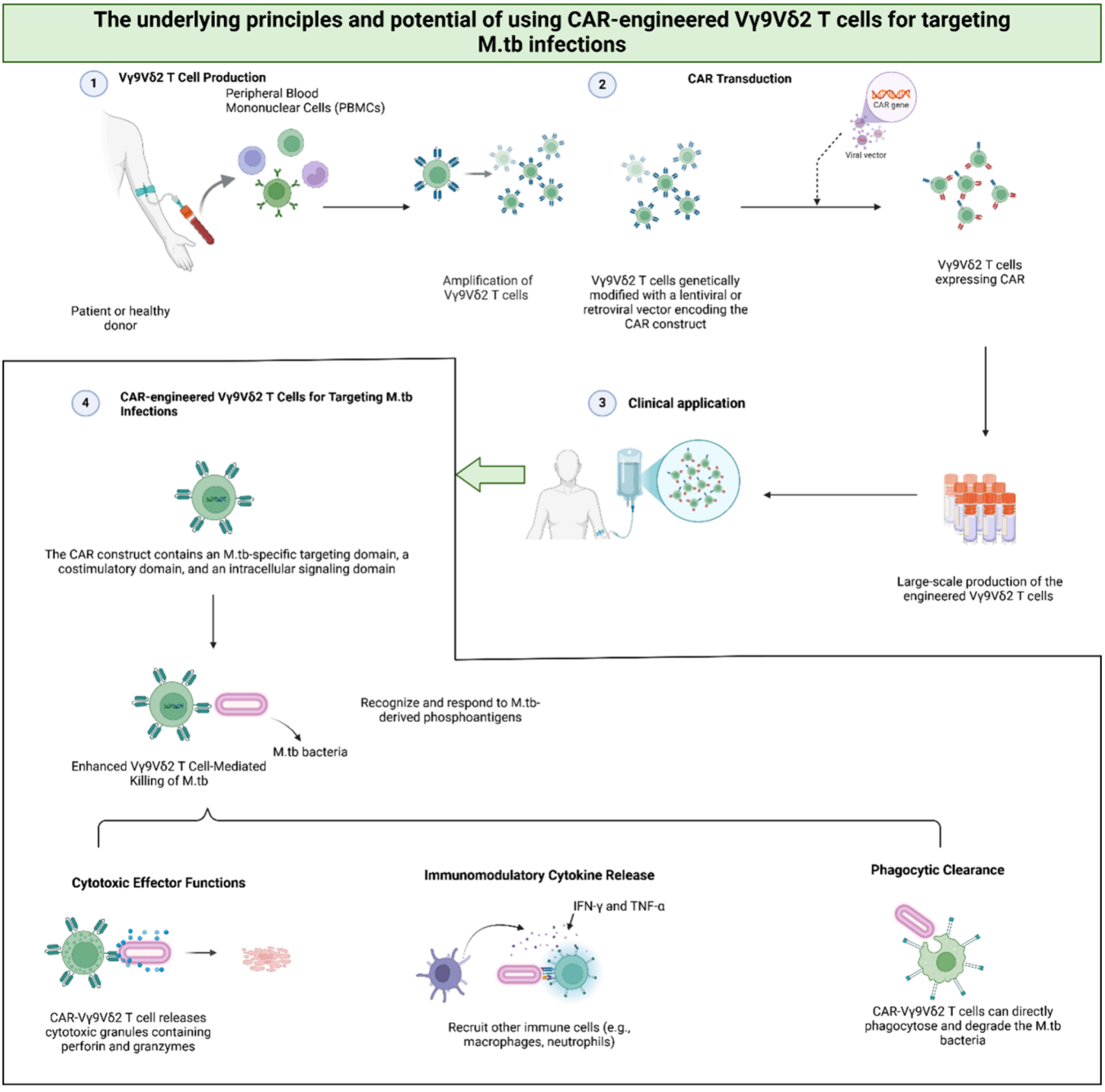

Figure 4. T cell-based immunotherapy can help treat persistent *Mycobacterium tuberculosis* (M. tb) infections. T cells were isolated from donors with M. tb infection and expanded ex vivo (1). The cells are genetically engineered to express a chimeric antigen receptor (CAR) on their surface (2). CAR targets a selective antigen expressed only in M. tb-infected cells, not in healthy cells. CAR-modified Vγ9 Vδ2 T cells are infused back into the patient (3). Their CAR enables the recognition of M. tb-infected cells presenting the target antigen. Once activated via CAR signaling, engineered T cells selectively seek out and destroy M. tb-harboring cells through targeted immune responses, such as cytokine release and cytotoxic granule pathways (4).

Regulatory Hurdles in mAbs Development. A critical shortcoming in previous mAb development efforts has been the advancement of candidates into clinical trials without adequate preclinical validation using clinically relevant assays. The pressing need to address antibiotic resistance has sometimes led to accelerated development programs that bypass essential foundational research. Many programs have lacked robust predictive *in vitro* and *in vivo* models that could establish proper dosing parameters, identify responsive patient subgroups, and verify the therapeutic efficacy. This oversight has frequently resulted in clinical trials with poorly defined patient selection criteria or inappropriate outcome measures. For instance, patients with advanced comorbidities may exhibit diminished responses to mAb therapy, whereas those with elevated inflammatory markers might experience exaggerated immune reactions. These variables underscore the importance of thorough preclinical characterization before clinical evaluation.

To improve the success rate of mAb therapies for bacterial infections, future development programs must emphasize three key elements: comprehensive target validation, detailed mechanistic studies, and the implementation of predictive

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assays early in the discovery process. Target validation should include the assessment of antigen conservation across clinical isolates, expression during human infection, and accessibility to antibody binding. Mechanistic studies must define how mAbs neutralize pathogens or modulate immune responses, including potential effects on complement activation, opsonophagocytosis, and toxin neutralization. Finally, developing clinically predictive assays, including advanced *in vitro* systems and animal models that better recapitulate human disease, will be essential for selecting the most promising candidates. By addressing these factors systematically, researchers can enhance the probability of clinical success while minimizing patient risks.

Variable Efficacy of mAbs in Immunocompromised Patients. Clinical trials of mAbs targeting bacterial endotoxins have revealed significant variability in efficacy, especially among immunocompromised patients. Although preclinical studies have demonstrated the ability of these mAbs to neutralize lipopolysaccharide (LPS)-induced inflammatory pathways, their clinical application has been limited by individual differences in immune system function.[73,74] Patients with compromised immunity, such as those with HIV/AIDS, malignancies, or post-transplant immunosuppression, frequently demonstrate impaired antibody-dependent immune mechanisms that may diminish the therapeutic potential of mAbs. These patients are also vulnerable to opportunistic infections when antibody-based interventions disrupt their precarious immune balance. Notably, opportunistic Gram-negative pathogens such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa* commonly cause treatment-resistant infections in this susceptible population.

The challenges observed in these clinical trials emphasize the complex relationship between mAb therapies and host immunity. While designed to target specific bacterial components, the drugs' ultimate effectiveness depends heavily on the recipient's immune competence. This dependency creates particular obstacles for immunocompromised patients, whose impaired immune function may not adequately support the intended therapeutic mechanisms, highlighting the need for more personalized therapeutic approaches that account for the underlying immune status. These findings suggest that future mAb development should incorporate immune function assessments to better predict treatment responses across diverse patient populations.

Future Directions in Bacterial Antibody Development. Despite recent efforts in bacterial mAb development, much remains to be addressed, and future works are needed to fast-track these efforts using novel technologies. Artificial intelligence (AI) has emerged as a transformative force in antibody design and discovery, offering unprecedented capabilities to accelerate the development of novel therapeutics. AI, especially through machine learning (ML) and deep learning, has enhanced the precision and efficiency of antibody discovery by leveraging large data sets for structure prediction, binding prediction, and developability assessments.[76] These advancements have been pivotal in overcoming traditional challenges in antibody design, such as the lack of accurate structural data for antibodies and antigens. AI's integration into this field has streamlined the discovery process and improved the accuracy of predicting antibody properties and interactions. Most importantly, AI reduces the time and cost associated with antibody discovery by automating and optimizing various stages of the development process (Figure 3).

Nanotechnology presents innovative strategies to enhance antibody delivery and efficacy, particularly through the utilization of nanoparticles. These nanoparticles can be engineered to interact effectively with microorganisms, causing detrimental alterations in their morphology and structure. This interaction is facilitated by the unique properties of the nanoparticles, such as their shape, size, and surface chemistry, which can be tailored to improve the targeting and delivery of antibodies. Modifying these properties allows nanoparticles to disrupt microbial structures, enhancing the antimicrobial efficacy of the antibodies that they deliver. For instance, nanophotothermal therapy utilizing inorganic nanoparticles, such as gold nanoparticles (AuNPs), leverages the unique photothermal properties of AuNPs to generate localized heat upon light irradiation, effectively killing bacteria. AuNPs can penetrate and disrupt biofilms. This capability is crucial for treating chronic infections like those caused by *Streptococcus mutans* and *Staphylococcus aureus*[77] Moreover, the orientation of the antibodies on the nanoparticles is crucial for maximizing antigen binding. Techniques like DNA-PAINT imaging help researchers understand and optimize this orientation, which is essential for effective targeting and immune cell engagement. Different conjugation strategies can modulate the exposure of antibody domains, such as Fab and Fc, which can be tuned for specific applications, enhancing the biological performance of the nanoparticles.

CELLULAR-BASED IMMUNOTHERAPY

Cellular immunotherapy involves the use of immune cells to treat diseases, including infections. This can be achieved through various approaches, including adoptive cell transfer, where immune cells are collected, expanded, and activated *ex vivo* before being infused into the patient (Figure 4). Alternatively, cellular immunotherapy can involve the *in vivo* modulation of immune responses to enhance the activity of specific immune cell populations. Cellular immunotherapy can employ various mechanisms to target and eliminate bacterial infections mediated by specialized cells. For instance, natural killer (NK) cells and cytotoxic T lymphocytes (CTLs) can eliminate bacteria-infected cells by releasing cytotoxic granules or activating the death receptors present on the surface of the target cell. This process plays a crucial role in eradicating intracellular bacteria shielded from antibodies and antibiotics. NK cells are recognized for their ability to secrete diverse cytokines, such as IFN-γ and TNF-α, which play a crucial role in activating macrophages and neutrophils, thereby enhancing their bactericidal functions. T cells, particularly the CD4+ and CD8+ subsets, are pivotal in producing cytokines that regulate the immune response. For example, CD4+ T cells can generate IL-2, which is a key factor in the proliferation and activation of T cells. Furthermore, cytokines can stimulate inflammation, which assists in recruiting immune cells to the site of infection and aids in the eradication of bacteria.

Macrophages and B cells act as antigen-presenting cells (APCs), presenting bacterial antigens to T cells and triggering an adaptive immune response. This intricate process includes the internalization and processing of bacterial antigens, followed by the presentation of antigenic peptides on MHC molecules to T cells. Neutrophils can release neutrophil extracellular traps (NETs), which are intricate structures of histones, antimicrobial proteins, and DNA. These NETs can ensnare and eradicate bacteria, impeding their spread and facilitating their eradication. Nevertheless, an overabundance of NET formation can also lead to tissue injury and inflammation. Below are some specific

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cellular-based immunotherapeutics recently developed to combat ESKAPE pathogens.

Adoptive Cell Therapy. Adoptive cell therapy (ACT), often called adoptive T cell therapy, is a form of immunotherapy involving the infusion of immune cells, specifically T cells, into a patient's body to treat a range of diseases. One auspicious approach in ACT is chimeric antigen receptor (CAR)-T cell therapy. This therapy includes the genetic modification of the patient's T cells. By replacing the native T cell receptor with a CAR that can recognize specific antigens independently of the MHC, the engineered CAR-T cells can proliferate in significant quantities before being reintroduced into the patient to assist in combating infections (Figure 4). By directly recognizing pathogens, CAR-T cells can overcome the limitations of conventional T cell responses.

In their study, Liang and co-workers[79] revealed new possibilities for treating tuberculosis (TB) through genetically modified T cells expressing a CAR that explicitly targets the Vγ9 Vδ2 subtype of T cells. These CAR-expressing Vγ9 Vδ2 T cells were engineered to identify a specific antigen on M. tb-infected cell surfaces, enabling them to selectively target and eliminate infected cells while preserving healthy ones. Genetic modification of T cells enhances their cytotoxic capabilities and immune responses to M. tb. Encouragingly, the results showed that the Vγ9 Vδ2 T cell approach was clinically safe for TB immunotherapy and appeared to provide clinical benefits in multiple areas, such as promoting the repair of lung lesions, improving immune responses, and helping to reduce the mycobacterial load. These effects were observed when the cells were treated with or without anti-TB medication. CAR-resistant Vγ9 Vδ2 T cells are manufactured in large quantities from healthy donors and can be administered to multiple patients. This eliminates the need for individualized cell therapy preparations and provides a more scalable and cost-effective treatment option.

Cytokine-Induced Killer (CIK) Cell Therapy. In addition to CAR-T cell therapy, a case report described an MDR-TB patient who did not respond well to the standard WHO-directed treatment regimen. Despite aggressive therapy with second-line antibiotics, the patient remained culture-positive for acid-fast bacilli, indicating an active infection. To improve her outcomes, she received a novel immunotherapy called cytokine-induced killer (CIK) cell therapy and antibiotic treatment. The CIK cells were administered in eight courses and adjunctively with her standard MDR-TB medications. The findings suggested that CIK immunotherapy holds promise as a supplemental treatment to enhance the effectiveness of second-line antibiotic regimens for patients with MDR-TB. Subsequent research evaluated the clinical outcomes in MDR-TB patients by comparing combined CIK-cell immunotherapy with standard antibiotic therapy.[80] The results showed higher conversion rates of the sputum and culture tests in the combination group. The patients also experienced better symptom relief, better lesion absorption on imaging, and higher overall recovery rates. Additionally, the monitoring of serology and immunological markers demonstrated that CIK treatment had a good safety profile. This case report provides early evidence that CIK-cell immunotherapy may be a valuable adjunct for improving outcomes in patients with MDR-TB when it is added to standard antibiotic regimens.

Engineered and Enhanced Macrophages. Researchers have created CRV peptide-modified lipid nanoparticles capable of delivering CAR mRNA and CASP11 siRNA intracellularly to macrophages.[81] This allowed the transient *in situ* reprogramming of macrophages by expressing a CAR targeting MRSA along with knocking down an evasion factor. Genetically engineered macrophages can be generated directly at the site of infection. Tests showed that the modified macrophages had an enhanced ability to phagocytose and digest MRSA intracellularly. This novel nanoparticle-based strategy can empower the immune system to overcome superbug infections such as MRSA by preventing bacteria from evading immune clearance inside macrophages. The ability to transiently program macrophages *in situ* using mRNA and siRNA delivery represents an innovative therapeutic approach.

An even more fascinating method is the use of a nanoparticle coating containing therapeutic genes to generate enhanced macrophages with targeted bacterial-killing abilities. For instance, when introduced into mouse macrophages via nanoparticles, the genes encoding CARs specific for *Staphylococcus aureus* and shRNAs against caspase-11 allowed the cells to become "super CAR-MΦs", which are macrophages with improved antibacterial functions. Specifically, caspase-11 shRNA promoted the mobilization of macrophage mitochondria around phagosomes containing ingested *S. aureus*. This mitochondrial recruitment generated reactive oxygen species with potent bactericidal properties. *In vivo* analysis revealed that these modified macrophages could eliminate *S. aureus* infections at the interface between the bone and surgical implants.[82]

Additionally, Wang and colleagues[83] developed a novel therapeutic approach involving the adoptive transfer of macrophages loaded with a near-infrared photosensitizer called Lyso700D. The photosensitizer can be specifically taken up by macrophage lysosomes. This approach was intended to boost the immune response and allow macrophages to use their natural ability to track, capture, and destroy bacteria via phagocytosis. Directly delivering photosensitizers to lysosomes containing engulfed bacteria maximized the photodynamic effect while minimizing potential side effects in other tissues.

Following these, a recent study have uncovered the critical regulatory functions of mmu-miR-25−3p in macrophage autophagy and its influence on intracellular *Mycobacterium bovis* BCG survival.[84] Bioinformatics analysis identified mmu-miR-25-3p as a differentially expressed microRNA in BCG-infected macrophages, with DUSP10 as its target gene serving as a key autophagy regulator. The study demonstrated that BCG infection upregulated mmu-miR-25-3p while downregulating DUSP10, resulting in increased expression of autophagy markers such as Atg7, Beclin1, LC3-II, and Atg5. Furthermore, the overexpression of mmu-miR-25-3p or the silencing of DUSP10 led to the activation of ERK1/2 phosphorylation, a critical MAPK signaling pathway that further promoted autophagy in macrophages. This enhanced autophagy, driven by the mmu-miR-25-3p/DUSP10 axis, significantly reduced the BCG intracellular survival, as indicated by reduced bacterial colony-forming units. These results suggest that mmu-miR-25-3p holds potential as a target for immunotherapy against tuberculosis and for creating drug delivery systems based on exosomes.

Building on strategies to combat intracellular *Mycobacterium tuberculosis* (M. tb), a novel macrophage-targeted nanodecoy system utilizing iron oxide nanoparticles (IONPs), named IONPs-PAA-PEG-MAN, was developed to enhance innate immunity and improve drug efficacy against intracellular M. tb.[85] This nanodecoy demonstrated preferential uptake by macrophages through mannose receptor-mediated endocytosis and phagocytosis, leading to significantly higher intracellular

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accumulation compared with nontargeted IONPs. The encapsulation of the antibiotic rifampicin within the nanodecoy (Rif@IONPs-PAA-PEG-MAN) enabled pH-sensitive drug release, maintaining sustained rifampicin levels in infected macrophages and promoting colocalization with intracellular M. tb. The nanodecoy effectively surrounded M. tb-containing phagosomes or colocalized in the same lysosome, ensuring direct exposure of the bacteria to the drug. Additionally, Rif@IONPs-PAA-PEG-MAN polarized infected macrophages toward an M1 antimycobacterial phenotype, enhancing pro-inflammatory TNF-α production while decreasing anti-inflammatory IL-10 levels. *In vitro* and *ex vivo* experiments demonstrated that Rif@IONPs-PAA-PEG-MAN significantly improved the killing of intracellular M. tb in infected macrophages and monocyte-derived macrophages compared with free rifampicin or nonencapsulated IONPs. In an acute M. tb infection mouse model, treatment with Rif@IONPs-PAA-PEG-MAN reduced lung bacterial burden and mitigated M. tb-driven inflammation without causing notable toxicity. Additionally, the nanodecoy's ability to colocalize with intracellular M. tb and expose it to both rifampicin and excessive iron further amplified its bactericidal effects. By combining macrophage-specific targeting with dual mechanisms of action, this nanodecoy system represents a promising candidate for more effective tuberculosis therapy, particularly against drug-resistant strains.

Engineered Neutrophils. Researchers have developed an innovative nanoparticle immunotherapy platform that significantly boosts the neutrophil activity against *Staphylococcus aureus* infections.[86] The nanoparticles combine red blood cell membranes, the antifungal drug naftifine, and hemoglobin (Hb) for multimodal therapeutic effects. *In vitro* experiments showed that the nanoparticles were effective against *S. aureus* persisters, planktonic cells, and biofilms. The nanoparticles enhanced neutrophil antimicrobial function under hypoxic conditions. In mouse models of MRSA peritonitis, pneumonia, thigh infection, and bacteremia, the nanoparticles demonstrated excellent therapeutic efficacy, reducing bacterial burdens and alleviating infection-associated inflammation. The multimodal design of the nanoparticles, which engage both bacterial virulence factors and host immune defenses, makes this a promising approach for combating antimicrobial-resistant *S. aureus* infections. The enhanced neutrophil function is a key mechanism underlying therapeutic effects.

In another effort, a breakthrough therapeutic approach was developed by coupling fusidic acid-loaded nanoparticles with neutrophil-directed roflumilast carriers in a single integrated system. This approach enabled simultaneous attack against bacteremia-induced inflammation and MRSA infection. Compared with the nontargeted versions or free drugs, the functionalized nanosystem more strongly suppressed cytokine and chemokine overexpression. In addition, the median survival time of MRSA-infected animals was extended from 50 to 103 h without any observed toxicity.[87]

Overall, the field of adoptive cell therapy for bacterial infections is rapidly evolving, with promising strategies involving CAR-T cells, CIK cells, engineered macrophages, and macrophage-targeted nanomedicines. These approaches harness and enhance innate and adaptive immune responses to combat persistent and drug-resistant bacterial infections.

Limitations and Challenges of Cellular-based Immunotherapies. Efficient delivery and homing of therapeutic immune cells to the infection site pose a significant challenge. Systemic administration of immune cells may result in limited penetration into infected tissues, thereby reducing the therapeutic efficacy. Strategies to enhance immune cell trafficking and infiltration into infected sites are needed to improve the effectiveness of cellular immunotherapies. Moreover, cellular-based immunotherapies present a potential risk of off-target effects and immunopathology. The unregulated activation of immune cells may result in heightened inflammation and tissue injury. Precise targeting and regulation of immune cell activity are essential to minimize these risks. Additionally, the high costs and complex manufacturing processes of cellular immunotherapies limit their accessibility, particularly in resource-limited settings. Therefore, streamlining the manufacturing processes and reducing production costs are crucial for broader applications.

Future Directions in Cellular-based Immunotherapies. Despite recent efforts in the development of cellular-based immunotherapies, much remains to be addressed, and future works are needed to boost these efforts using novel technologies. T cell-based therapies are being explored to combat infectious diseases, underscoring the importance of T cells in adaptive immunity. The innovative CAR-T cell therapy, renowned for its remarkable achievements in cancer treatment, shows great potential for application in combating bacterial infections. By engineering CAR-T cells to identify bacterial antigens and eliminate infected cells,[79] a new avenue for treatment has emerged. Advances in genetic engineering and synthetic biology have also enabled the development of engineered immune cells, such as cytokine-induced killer (CIK) cells, which have shown efficacy in enhancing chemotherapy against MDR tuberculosis.[80] Similarly, the mass production of iPSC-derived macrophages in bioreactors represents another innovative approach, providing a scalable method to generate therapeutic phagocytes for treating bacterial airway infections. For instance, human iPSC-derived macrophages (iMΦs) have been evaluated as a potential therapy for pulmonary *S. aureus* infections.[88] iMΦs exhibited efficient phagocytosis and antimicrobial activity against both methicillin-sensitive and methicillin-resistant *S. aureus* strains *in vitro*. Adoptive transfer of iMΦs into the lungs of immunodeficient mice with *S. aureus* pneumonia significantly reduced the bacterial burden, lung inflammation, and tissue damage compared to untreated infected controls. Moreover, developing novel delivery systems, such as nanoparticles and outer membrane vesicles (OMVs), can improve the targeting and efficacy of cellular immunotherapies. For example, rationally designed nanoparticles have been shown to successfully overcome delivery barriers and shape adaptive immunity. Biomaterials with robust packaging capabilities are also being explored to enable sustained and localized drug release at the target site.

IMMUNOMODULATORY APPROACHES

Another emerging approach to combating ESKAPE pathogens is the use of immunomodulators. This strategy harnesses the body's inherent mechanisms to amplify therapeutic advantages. The immune system is vital for defending against infections and maintaining equilibrium. However, an exaggerated immune reaction can trigger persistent inflammation, paving the way for various diseases. Immunomodulation involves fine-tuning of the immune response. It strives to reestablish balance by lessening an overactive response or bolstering a feeble response Immunomodulators encompass a diverse group of agents capable of regulating the host's immune response to improve

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bacterial elimination. Immunomodulators exert their effects through various mechanisms depending on the specific agent and the target immune pathway. Some immunomodulators enhance the activity of immune cells, such as macrophages and neutrophils, promoting phagocytosis and bacterial killing. Other immunomodulators modulate cytokine production, reducing tissue damage and excessive inflammation. Additionally, some immunomodulators enhance the adaptive immune response, promoting the development of long-lasting immunity. Immunomodulators can be used as adjunct therapies to antibiotics or standalone treatments for drug-resistant infections, offering a flexible approach to managing these complex conditions.

Research, Case Studies, Preclinical and Clinical Trials.

Modulation of Innate Immune Responses. Some molecules have been found or harnessed to modulate the immune response against bacterial infections. For instance, pentraxin 3 (PTX3), an intriguing protein, possesses diverse roles in inflammation and immune regulation. PTX3 is crucial in regulating the innate immune response during bacterial infections. In mouse models, *Streptococcus suis* serotype 2 (SS2) strain HA9801 was shown to substantially enhance inflammatory responses, with simultaneous PTX3 administration further intensifying this effect through increased inflammatory cell recruitment and elevated IL-6 production.[89] Additionally, PTX3 was found to enhance the phagocytosis of the SS2 HA9801 strain by macrophages. Most notably, supplementation with exogenous PTX3 significantly reduced bacterial loads in the liver, lungs, and blood of SS2-infected mice in a dose-dependent manner compared with those of HA9801-infected mice alone. This suggests that in the event of an SS2 infection, PTX3 might help remove bacteria by boosting the host's inflammatory response. For a strong inflammatory response, both PTX3 and capsular polysaccharide SS2 (CPS2) are required.

Also, antimicrobial defense peptides have emerged as a promising new class of therapeutic agents against drug-resistant pathogens. Recently, the immunomodulatory capabilities of these peptides have started to gain recognition. For instance, the peptide lactomodulin, produced by *Lactobacillus* species, has been identified as a novel therapeutic agent with dual anti-inflammatory and antimicrobial properties.[90] Lactomodulin demonstrated dual therapeutic effects by significantly reducing key proinflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-8) while maintaining potent antimicrobial activity against MDR pathogens, including MRSA and VRE. These properties position it as a promising combined anti-inflammatory and antimicrobial therapeutic. Furthermore, recent studies have examined the function of the cathelicidin-related antimicrobial peptide (CRAMP) in host defense against MDR *Acinetobacter baumannii*.[91] Mice lacking the CRAMP gene were intranasally infected with *A. baumannii* compared to wild-type mice. CRAMP knockout mice exhibited increased bacterial counts in the lungs and decreased recruitment of immune cells, including neutrophils, to the site of infection. The levels of proinflammatory cytokines IL-6 and CXCL1 were lower in CRAMP-deficient mice; however, there was a greater concentration of the anti-inflammatory cytokine IL-10. *In vitro*, neutrophils from knockout mice showed an impaired ability to phagocytose and kill bacteria compared to wild-type neutrophils. CRAMP was also found to regulate cytokine and chemokine production in neutrophils exposed to *A. baumannii*. Additionally, signaling pathways involved in the immune response were disrupted in neutrophils lacking CRAMP. These findings underscore the importance of CRAMP in orchestrating the immune response and neutrophil function in infections with MDR pathogens.

Modulation of Mesenchymal Stem Cells (MSCs). MSCs have shown promise in fighting infections through their antimicrobial peptide secretion and ability to recruit immune cells such as monocytes and neutrophils. When activated by TLR-3, a receptor found on immune cells, MSCs from humans, dogs, and horses exhibit enhanced bacterial killing of *Staphylococcus* biofilms in laboratory and animal studies. A clinical study revealed that administering TLR-3-activated MSCs along with vancomycin improved outcomes for horses with induced septic arthritis more than antibiotics alone.[92] Researchers have investigated whether a MSC-conditioned medium (MSC-CM) can be used as an adjuvant therapy for antibiotics. For instance, recent *in vitro* studies have demonstrated that a mesenchymal stem cell-conditioned medium (MSC-CM) exhibits both antibacterial and immunoregulatory effects on *P. aeruginosa*-infected human corneal epithelial cells (HCECs).[93] Bacterial growth assays revealed that MSC-CM had substantially greater antibacterial activity against *P. aeruginosa* than the control treatments. Notably, MSC-CM inhibited the production of antimicrobial peptide lipocalin 2 and proinflammatory cytokines TNF-α and IL-6 by bacterial lipopolysaccharides in HCECs. The levels of lipocalin 2, TNF-α, and IL-6 were also moderately controlled by the combination of MSC-CM and ciprofloxacin.

Modulation of Metabolic Pathways. Another classical way scientists combat ESKAPE pathogens is via metabolic pathway modulation. For instance, the cholesterol 25-hydroxylase (Ch25h) enzyme has been demonstrated to significantly modulate the body's response to *S. pneumoniae* infection.[94] Research has revealed important connections between cholesterol metabolism and immune function, demonstrating its role in regulating inflammatory cytokine production and bacterial clearance in both wild-type and Ch25h-deficient mice.[94] During *S. pneumoniae* infection, Ch25h initiates and controls chemokine and cytokine production in the lungs. Mice exhibited improved phagocytosis and bacterial clearance when Ch25h was not present.

In addition, epigenetic modifications caused by mycobacterial infections can affect the immune response. Comparative epigenomic profiling of leprosy patient skin identified disease-associated DNA methylation signatures absent in healthy individuals.[95] Further analysis revealed a connection between leprosy susceptibility and the T helper 17 cell development pathway. Integrated data on methylation, gene expression, and genome-wide association analysis revealed that IL-23R, a specific gene in this pathway, is crucial for protecting against mycobacteria. Laboratory experiments showed that through the IL-23/IL-23R system, macrophages can better clear *Mycobacterium tuberculosis* by inducing a specialized programmed cell death process. This pathway promotes the development of T helper 17 cells, which secrete proteins that enhance the immune attack during infection. Mice lacking the IL-23R gene exhibit a reduced ability to fight *Mycobacterium* spp.

Modulation of Metal Ion Homeostasis. Recently, some groups have harnessed metal ions to tackle ESKAPE pathogens due to their distinctive properties. For instance, copper (Cu) serves as a cofactor for enzymes engaged in defending against oxidative stress and regulating the immune system. It bolsters the innate immune system's capacity to fend off bacterial infections. The host cells infected by bacteria actively acquire copper from their cytoplasm. Accumulated Cu increases the ability of cells to combat invading pathogens, including

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intracellular and extracellular bacteria. Recent research suggests that copper operates as a signaling molecule that controls the kinase activity of α-kinase 1 (ALPK1), a cytosolic pattern recognition receptor. Copper directly binds to ALPK1, which is crucial for its enzymatic function. This binding amplifies ALPK1's responsiveness to the bacterial metabolite ADP-heptose, leading to a heightened immune reaction from the host cell against bacterial infections. Li and colleagues recently demonstrated that copper-treated fish exhibit greater cytokine production, increased recruitment of immune cells, and better bacterial clearance than untreated fish during infection.[96]

Also, breakthrough wound dressing incorporating copper−zinc bioactive glass nanoparticles in a bilayer hydrogel matrix demonstrated precise immunomodulation for treating MRSA-infected wounds.[97] The bilayer bioactive glass structure consists of a copper-doped outer layer and a zinc-doped inner layer, which can sequentially release the corresponding metal ions to regulate the immune response. The hydrogel matrix loaded with bioactive glass provides a moist microenvironment and gradual release of metal ions, guiding the spatiotemporal modulation of inflammation and tissue regeneration. *In vitro* experiments demonstrated that the Cu/Zn-doped bioactive glass hydrogel inhibited MRSA growth, decreased proinflammatory cytokine production, and promoted skin cell proliferation and migration. In a mouse model of MRSA-infected wounds, the Cu/Zn-doped bioactive glass hydrogel enhanced wound closure, reduced the bacterial burden, and shifted the inflammatory response from a pro-inflammatory state to an anti-inflammatory state. This Cu/Zn-doped bioactive glass-hydrogel composite is a promising multifunctional wound dressing that can harness the immunomodulatory properties of metal ions to support the healing of MRSA-infected wounds.

Multifunctional Nanoplatforms for Immunomodulation. Multifunctional nanoplatforms integrating antimicrobial, immunomodulatory, and other therapeutic components are emerging as innovative solutions to combat intracellular bacterial infections. One such development employs a cascade-targeted approach that combines antibiotics, reactive oxygen species (ROS), nitric oxide (NO), and immunotherapy to achieve synergistic effects. This nanoplatform enhances bacterial clearance and stimulates the host immune response by promoting the secretion of proinflammatory cytokines, including TNF-α and IL-6, from macrophages. In macrophages infected with MRSA, the cascade-targeted nanoplatform demonstrated superior bacterial killing compared with the individual components or nontargeted controls.[98] These results highlight the potential of this agent as a groundbreaking approach to eliminating intracellular pathogens while simultaneously boosting immune defenses.

Another innovative nanoparticle-based therapy was demonstrated to target biofilm-infected wounds by addressing both bacterial eradication and immune modulation.[99] This system integrated a photosensitizer, a quorum sensing inhibitor, and an NF-κB signaling pathway inhibitor, all encapsulated within a lipid−polymer hybrid nanoparticle. Upon near-infrared light irradiation, the photosensitizer generated ROS to disrupt bacterial biofilms, whereas the quorum sensing inhibitor suppressed biofilm formation. Simultaneously, the NF-κB inhibitor modulates the immune response, shifting it from a pro-inflammatory state to an anti-inflammatory state to facilitate wound healing. *In vitro* experiments demonstrated the ability of nanoparticles to kill biofilm-associated bacteria, inhibit quorum sensing, and reduce proinflammatory cytokine production by macrophages. In a mouse model of biofilm-infected wounds, the nanoparticles accelerated wound closure, decreased the bacterial burden, and mitigated inflammation more effectively than the controls. This multimodal therapeutic approach, which combines phototherapy, quorum sensing inhibition, and immunomodulation, represents a promising strategy for treating chronic biofilm-associated wound infections.[99]

Modulation of T Cell Responses. Modulating T cell responses is emerging as an innovative solution to combat intracellular bacterial infections. For instance, Bromley and colleagues[100] investigated the impact of CD4+ T cells on reshaping the cellularity of granulomas and regulatory networks following reinfection with *Mycobacterium tuberculosis* (M. tb). Upon reinfection with M. tb, CD4+ T cells modify the cellular makeup and gene regulatory networks within granulomas, leading to changes in the immune environment compared to those of the initial M. tb infection. Reinfection-driven changes in granuloma cellularity include increases in neutrophils, B cells, and CD4+ T cells along with decreases in the number of macrophages and dendritic cells. Transcriptomic analyses revealed that CD4+ T cells drive the remodeling of granuloma regulatory networks, upregulating immunomodulatory programs such as antigen presentation, lymphocyte activation, and cytokine signaling. The enhanced immunomodulatory state of the reinfection granulomas promotes more effective bacterial control than primary infection, potentially through T cell-mediated activation of other immune cells. These findings highlight the dynamic and adaptable nature of granulomas, which can be rapidly remodelled by CD4+ T cells to enhance immunity upon repeated M. tb exposure, providing insights into the host−pathogen interplay during tuberculosis.

In a related study aimed at understanding immune responses, an analysis of gene expression data revealed significant differences in the immune response to enteric infections between areas with high and low endemicity.[101] The analysis showed a pronounced suppression of GRB-2, a critical adaptor molecule in T cell receptor (TCR) signaling, as a primary immunomodulatory response in the endemic group. Further research indicates that the suppression of GRB-2 is linked to the inhibition of downstream TCR signaling pathways, potentially restricting T cell activation and proliferation in endemic regions. The study also observed a positive correlation between activated T cell regulators and mediators of Hedgehog signaling in the endemic population, suggesting a shift toward an effector T cell response rather than an inductive one. STAT3 was identified as a key transcription factor that negatively regulates TCR signaling while promoting Hedgehog signaling, indicating its role in determining the dual-phase functional state of T cells in endemic areas. The acute suppression of GRB-2 signaling highlights a potential regulatory mechanism that the immune system employs to control hyperactivation in frequently exposed populations, which needs to be considered in the design of region-specific vaccines. The findings provide insights into how baseline immunological profiles in endemic regions shape and modulate host responses to enteric infections, contributing to the suboptimal vaccine efficacy in these settings.

Overall, immunomodulation for the treatment of bacterial infections is a rapidly evolving field. Various strategies target different aspects of the immune system, including innate immunity, stem cells, metabolic pathways, metal ion homeostasis, and T cell responses. These immunomodulatory approaches harness the body's inherent mechanisms to enhance

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the fight against persistent and drug-resistant bacterial infections.

Limitations and Challenges of Immunomodulatory Therapy. The immune system constitutes an intricate interplay of cells, cytokines, and signaling pathways, and its response to bacterial infection is highly dynamic and context-dependent. This complexity complicates the prediction of how immunomodulatory interventions might influence the overall outcome of a disease. The same intervention may have different effects depending on the specific pathogen, the host's genetic background, the stage of infection, and other factors. Patients with bacterial infections are a heterogeneous group with varying degrees of immune competence, underlying medical conditions, and disease severity. This heterogeneity makes it difficult to identify the patient subgroups that are most likely to benefit from immunomodulatory therapy. Clinical trials of immunomodulatory agents often yield conflicting results due to differences in patient populations, study designs, and outcome measures.

Another major concern with immunomodulatory therapy is the risk of overstimulating the immune system, leading to excessive inflammation and tissue damage. Cytokine storms, characterized by the uncontrolled release of pro-inflammatory cytokines, can cause acute respiratory distress syndrome (ARDS), multiorgan failure, and death. Therefore, it is crucial to carefully balance the immunostimulatory and anti-inflammatory effects of immunomodulatory agents. Moreover, many immunomodulatory agents have broad effects on the immune system, which can lead to unintended consequences such as immunosuppression and increased susceptibility to secondary infections. For example, corticosteroids, which are commonly used to suppress inflammation, can also impair immune cell function, increasing the risk of bacterial infections. In addition, identifying reliable biomarkers to predict the treatment response and monitor the effects of immunomodulatory therapy remains a significant challenge. Biomarkers are needed to stratify patients, guide treatment decisions, and assess the efficacy and safety of immunomodulatory agents. However, the complexity of the immune system and the heterogeneity of patient populations make it difficult to identify sensitive and specific biomarkers.

Future Directions in Immunomodulatory Therapy. One of the most promising future directions is the development of more targeted immunomodulatory agents that selectively modulate specific immune pathways without causing broad immunosuppression. This can be achieved by targeting specific cytokines, receptors, or signaling molecules involved in the pathogenesis of bacterial infections. Nanotherapeutics offer an avenue for targeted immunomodulation. Encapsulating immunomodulatory agents within nanoparticles can enable selective delivery to specific immune cells or tissues, thereby reducing off-target effects and enhancing therapeutic efficacy.

Host defense peptides (HDPs), commonly known as antimicrobial peptides (AMPs), constitute a class of molecules possessing dual functionality: direct antimicrobial properties and immunomodulatory effects. These peptides can regulate the functions of various immune cells, including T cells, macrophages, dendritic cells, and neutrophils. Synthetic derivatives of HDPs, known as innate defense regulators (IDRs), exhibit immunomodulatory effects that are protective even in the absence of direct antimicrobial activity. For example, the synthetic IDR peptide 1018 demonstrates superior wound healing capabilities compared to natural host defense peptides LL-37 and HB-107, with evidence suggesting that these regenerative effects occur through mechanisms distinct from antimicrobial activity.[102] Structural modifications of the sequence, helicity, hydrophobicity, charge, and configuration of these peptides could optimize them for future clinical use.

Also, employing personalized medicine strategies that consider the unique characteristics of each patient could offer significant potential for enhancing the outcomes of immunomodulatory therapy. This involves identifying biomarkers that can predict the treatment response and tailoring the choice and dose of immunomodulatory agents to each patient's specific needs. For example, a systematic review and meta-analysis highlighted CXCL9 and CXCL10 as potential biomarkers for monitoring treatment responses in patients with pulmonary tuberculosis. They offer a noninvasive method to evaluate treatment efficacy, which is crucial given the limitations of traditional methods like sputum smear microscopy.[103] Moreover, studies have shown that INF-γ levels decrease significantly in patients who complete preventive TB treatment. This suggests its potential as a biomarker for monitoring treatment response, especially in high TB-burden areas.

Finally, combining immunomodulatory agents with antibiotics or other antimicrobial therapies may be more effective than using either approach alone. Immunomodulation can enhance the activity of antibiotics by improving immune cell function and reducing inflammation, while antibiotics can reduce the bacterial burden and prevent overstimulation of the immune system. Studies demonstrated that combining active vitamin D3 (vitD) and phenylbutyrate (PBA) can boost human macrophage defenses against MDR-TB through immunomodulation.[104] The vitD + PBA combination effectively suppressed the intracellular proliferation of clinical MDR-TB strains in human macrophages, showing additive effects with rifampicin (RIF) or isoniazid (INH). This treatment upregulated key antimicrobial effectors, including cathelicidin LL-37, β-defensin 1, and nitric oxide synthase, while inducing autophagy in infected macrophages. Remarkably, vitD + PBA combined with INH achieved MDR-TB growth inhibition comparable to a >125-fold higher INH dose alone, demonstrating potent synergistic activity. This study provides compelling evidence that immunomodulatory agents can enhance conventional treatment by activating multiple immune pathways, potentially contributing to the development of next-generation individualized treatment options.

POTENTIAL OF COMBINING IMMUNOTHERAPIES WITH OTHER ANTIMICROBIAL THERAPIES

Antibiotics. Immunotherapies can work synergistically with antibiotics by enhancing the host's immune defenses, targeting bacterial virulence factors, and modulating immune responses to reduce antibiotic tolerance. Immunotherapies can modulate the immune response to reduce the production of ROS by macrophages, which are known to induce antibiotic tolerance in bacteria like *Staphylococcus aureus*.[105] By shifting the macrophage response from a pro-inflammatory (M1-like) to an anti-inflammatory (M2-like) state, immunotherapies can improve the efficacy of antibiotics against intracellular pathogens such as *M. tuberculosis* and *Salmonella enterica* serovar Typhimurium.[106] Enhancing CD4+ T cell function has been explored as a strategy to improve antibiotic therapy in tuberculosis.[107] Immune-based strategies have been used to target nonessential gene products of *S. aureus*, disrupting virulence mechanisms and enhancing the host's immune

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defenses. This approach enhances the effectiveness of antibiotics in treating invasive staphylococcal diseases by decreasing pathogen survival and immunopathology.

Quorum Sensing Inhibitors. The integration of immunotherapies with quorum sensing inhibitors (QSIs) presents a promising strategy for addressing bacterial infections, particularly despite antibiotic resistance. Quorum sensing (QS) functions as a bacterial communication system that governs virulence, biofilm formation, and antibiotic resistance. By targeting QS, QSIs can reduce bacterial virulence without promoting resistance, making them attractive complements to immunotherapies. Natural plant extracts, such as those from *Syzygium aromaticum* and *Eucalyptus camaldulensis*, have shown anti-QS activity by reducing violacein formation and QS signal production in bacteria like *Pseudomonas aeruginosa*.[108] Synthetic derivatives, such as coumaperine from *Piper nigrum*, have demonstrated dual activity by inhibiting both QS and inflammatory pathways, such as NF-κB, which are often upregulated in bacterial infections. QSIs, such as halogenated furanones, have been shown to effectively inhibit biofilm formation in pathogens like *Pseudomonas aeruginosa* by disrupting autoinducer signaling.[109] Natural compounds, including terpenoids and flavonoids, have demonstrated anti-QS activity by inhibiting autoinducer release and gene expression. Further research is needed to optimize the combination of QSIs with immunotherapies, focusing on understanding the molecular interactions and potential side effects in human subjects.

Host-Directed Therapies. Immunotherapy enhances the immune system's capacity to combat infections, whereas host-directed therapies (HDTs) target host cellular processes to either support immune function or disrupt pathogen survival mechanisms. This combination can address the limitations of traditional antimicrobial treatments, especially in the context of drug-resistant infections and complex comorbid conditions like tuberculosis with diabetes or HIV. For example, all-trans retinoic acid (ATRA)-loaded nanoparticles have been developed to target TB by enhancing macrophage function, leading to reduced bacterial growth. Moreover, HDTs have been used to modulate the immune response, reducing inflammation and tissue damage while amplifying the effectiveness of current TB medications and thereby shortening the treatment duration. This approach helps to overcome drug resistance and improve patient outcomes. Using pan-caspase inhibitors such as quinoline−valine−aspartic acid−difluorophenoxymethyl ketone (Q-VD-OPH) has shown potential in reducing bacterial burden and lesion size in MRSA infections by modulating apoptosis pathways. Furthermore, recent studies have demonstrated that apoptotic body-like liposomes loaded with phosphatidylinositol 5-phosphate (ABL/PI5P), when combined with the lytic bacteriophage jBO1E specifically targeting KPC-producing multidrug-resistant *Klebsiella pneumoniae*, can significantly enhance macrophage-mediated intracellular bacterial killing.[110] The results indicated that the combined treatment effectively diminished intracellular and extracellular bacterial loads while modulating the inflammatory cytokine response. This suggests that this integrated approach could serve as a promising strategy for enhancing the clinical management of patients and mitigating the spread of MDR *Klebsiella pneumoniae* strains; however, further preclinical studies are necessary to address safety and efficacy concerns.

CLINICAL OUTCOMES AND LIMITATIONS OF IMMUNOTHERAPY AGAINST BACTERIAL INFECTIONS

Approved and Pipeline Immunotherapies. The number of approved immunotherapies specifically targeting bacterial infections remains limited. Currently, three mAbs are approved: raxibacumab, which targets the protective antigen of *Bacillus anthracis* for inhalational anthrax; obiltoxaximab, which neutralizes anthrax toxins by targeting the same protective antigen; and bezlotoxumab, which reduces the recurrence of infection. Additionally, more mAbs are in clinical trials, considered as standalone therapies or combined with antibiotics to improve efficacy. For example, MEDI4893 and AR-301 are human mAbs designed to target and neutralize the α-hemolysin Hla, a major virulence factor of *Staphylococcus aureus*. Arsanis is currently developing a combination of two mAbs, known as ASN100. This combination includes a Hla mAb (ASN-1) with cross-reactive properties against various toxins, along with other mAb (ASN-2) targeting the leukocidin LukAB. Similarly, XBiotech is currently in the process of testing a human mAbs (514G3) targeting the surface antigen staphylococcal protein A (SpA), with the aim of eliciting opsonophagocytosis. This mechanism involves the antibodies identifying surface elements, which is anticipated to lead to the clearance of bacteria. mAbs are generally well-tolerated, but potential adverse effects include hypersensitivity and infusion-related reactions. Therefore, mAbs require extensive preclinical and clinical testing to validate their efficacy and safety.

Existing treatments leverage immunomodulatory mechanisms, which can indirectly aid in combating bacterial infections. For instance, intravenous immunoglobulin (IVIG) is a blood-derived product containing pooled IgG antibodies obtained from various donors. IVIG is an approved product for treating various immunodeficiency disorders and inflammatory conditions. In bacterial infections, IVIG can provide passive immunity by neutralizing bacterial toxins, opsonizing bacteria for enhanced phagocytosis, and modulating the host's immune response. Regulatory approval for specific bacterial infection indications would require additional clinical trials demonstrating efficacy and safety.

Several immunotherapeutic strategies are being investigated for bacterial infections to enhance bacterial clearance and modulate the immune response. For example, IFN-γ is a cytokine that activates macrophages and improves their ability to kill intracellular bacteria. It has been investigated as a potential immunotherapy for infections such as tuberculosis.[111] Toll-like receptor (TLR) agonists bind to and activate TLRs, triggering the production of cytokines and chemokines to enhance the host's immune response. A synthetic TLR4 agonist, aminoalkyl glucosaminide 4-phosphate (AGP), also known as CRX-527, has been assessed for its potential in protecting melioidosis, an infection caused by *Burkholderia pseudomallei*. In a murine model, 66% of the mice administered AGP before a lethal intranasal challenge survived without exhibiting any signs of illness for 3 months. This protective effect was linked to a temporary elevation in the pulmonary levels of cytokines and chemokines, reinforcing the host's innate immunity and facilitating the rapid clearance of the bacteria.[112] TLR agonists can cause excessive inflammation and cytokine storms if not carefully regulated; therefore, they require careful dose optimization to balance efficacy and toxicity.

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Challenges in Translating Immunotherapies from the Bench to the Bedside. Despite promising results in preclinical studies, the translation of immunotherapeutic approaches to clinical applications has been limited. One of the primary limitations in preclinical research is the lack of standardized protocols. This variability extends to murine pneumonia models used to evaluate immunotherapeutic agents, making comparisons across studies difficult. This lack of standardized protocols results in variations in the animals' immune status, age, route of infection, and techniques utilized for sample processing. The European Innovative Medicines Initiative (IMI) is trying to address this by working toward a standardized, quality-controlled murine pneumonia model for efficacy testing. Future research should prioritize developing and validating standardized preclinical models that better reflect the complexities of human bacterial infections. This includes standardizing animal models, infection routes, and outcome measures. The goal is to strengthen the robustness and reproducibility of preclinical studies, thereby enhancing the translatability of the findings to clinical applications.

Methodological Shortcomings in Preclinical Research. Several issues in study design can limit the validity and applicability of preclinical findings. Many studies do not report sample size calculations or randomization procedures, and blinding procedures are often absent. These omissions introduce the risk of bias and can increase the apparent efficacy of the intervention. For instance, a systematic review and meta-analysis of preclinical studies using immune checkpoint inhibitors (CPIs) in sepsis models revealed significant heterogeneity across experiments.[113] The selection of appropriate animal models is crucial for preclinical research but can also be a significant limitation. Most studies are conducted in mice, which may not accurately reflect the complexities of human immune responses to bacterial infections. Additionally, the relevance of animal models to specific human diseases is not always well-defined. For instance, research in animal models of *Staphylococcus aureus* infection is hindered by a lack of comprehension regarding the host's immunological response to staphylococcal infection.

Complexities of Host−Pathogen Interactions. Bacterial infections entail an intricate interplay between the pathogen and the host's immune system. Preclinical studies often fail to fully capture this complexity, leading to an incomplete understanding of the therapeutic intervention's effects. The interplay between the innate and adaptive immune compartments is critical in determining the outcome of bacterial infections. Research efforts must integrate both immune compartments as a cohesive functional entity to enhance the effectiveness of immunotherapy across diverse diseases. Many real-world infections are polymicrobial and involve multiple bacterial species and other microorganisms. Preclinical studies often focus on monomicrobial infections, which may not accurately reflect the challenges of treating polymicrobial infections. The presence of multiple pathogens can alter the immune response of the host and the efficacy of immunotherapeutic interventions.

Biomarker and Microbiome-Related Challenges. A major obstacle in the development of immunotherapy is the absence of reliable biomarkers to predict and monitor treatment responses. Such biomarkers are crucial for patient stratification and therapeutic monitoring. Compounding this challenge is the recognition of the profound influence of gut microbiota on treatment outcomes. As a key modulator of immune function, the microbiome significantly affects host responses to immunotherapy; however, this relationship remains poorly understood. These knowledge gaps underscore the need for more comprehensive research into host-specific factors that dictate treatment success, including individualized microbiome profiles and other personalized medicine approaches to optimize immunotherapy efficacy.

Economic Challenges in Immunotherapeutic Production. The manufacturing of immunotherapeutic agents presents substantial economic barriers that influence their global availability and practical implementation. Complex production requirements for immunotherapeutic agents, particularly mAbs, drive elevated costs due to specialized bioreactor systems, rigorous purification protocols, and exacting quality assurance standards. These molecules require carefully controlled environments to preserve their structural integrity and biological functionality, necessitating significant investments in infrastructure and technical expertise. Unlike conventional pharmaceuticals, mAbs require living-cell-based production systems and compounding expenses.

These steep production costs translate into substantial pricing challenges, particularly for low-resource regions and underserved patient populations. The resulting financial barriers may intensify healthcare inequities as costly immunotherapies risk becoming accessible only to well-funded healthcare systems or affluent patients. This disparity is especially problematic in developing nations, where competing public health priorities and limited budgets restrict the adoption of high-cost therapies. Even in wealthier healthcare systems, payers increasingly demand clear demonstrations of value before they approve reimbursement for these expensive treatments. The premium pricing of immunotherapies necessitates a rigorous evaluation of their therapeutic value relative to that of conventional alternatives. Health economists emphasize the importance of comparative effectiveness research, particularly for treatments offering incremental rather than transformative clinical benefits. Parameters such as quality-adjusted life years (QALYs), treatment durability, and patient subgroup responsiveness become critical in cost−benefit analyses. Policymakers and formulary committees increasingly rely on health technology assessment (HTA) frameworks to guide funding decisions. This ensures that finite healthcare resources are allocated to interventions that demonstrate a meaningful clinical and economic value. This value-based approach helps to balance innovation incentives with sustainable healthcare expenditures while addressing ethical concerns about equitable treatment access.

Scalability Challenges in Immunotherapy Implementation. The transition of immunotherapies from research settings to widespread clinical use faces significant scalability challenges across multiple dimensions. Cell-based therapies require sophisticated equipment for cell isolation, expansion, genetic engineering, and stringent environmental controls throughout manufacturing. For instance, personalized cell-based treatments like CAR-T therapy present particularly complex manufacturing challenges due to their patient-specific nature. Each therapeutic batch requires individual cell processing, genetic modification, and rigorous quality testing processes that are inherently difficult to standardize and automate.

Equally challenging is the need for specialized healthcare professionals capable of safely administering these therapies and managing potential complications like cytokine release syndrome. The combined requirements for advanced technol-

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ogy and specialized training create a "last mile" problem in delivering these treatments to patients outside major medical centers. Moreover, the successful deployment of advanced immunotherapies depends heavily on specialized healthcare infrastructure that is unevenly distributed globally. Many regions lack the necessary facilities for producing and administering these treatments, including GMP-compliant cell processing centers and specialized treatment units. The shortage of clinicians and technicians trained in cellular therapy protocols creates additional implementation barriers, particularly in developing healthcare systems. These resource disparities result in significant geographic inequalities in patient access to cutting-edge treatments.

IMMUNOTHERAPY-RELATED CHALLENGES AND FUTURE DIRECTIONS

Overcoming Barriers to Effectiveness. Although much of the focus in immunotherapy research has been on host immune mechanisms, it is crucial to recognize the role of bacteria in developing immune resistance. During infection, pathogenic bacteria use potent techniques to control cell death, thereby preventing immune clearance from the host and creating favorable environments for multiplication. For instance, intracellular bacteria achieve this by delivering effectors that interfere with controlled cell death pathways through the type III secretion system (T3SS), which helps them avoid immune defenses. Just to mention a few, recent research has uncovered key mechanisms in *E. piscicida* pathogenesis, showing that the intracellular pathogen triggers PARP1-mediated death in mouse monocyte macrophages during infection.[117] Poly(ADP-ribose) (PAR) accumulates due to PARP1 activation, intensifying inflammatory signaling. However, *E. piscicida* developed a method to counter this defense mechanism. The T3SS uses a secretion system to deliver the effector protein YfiD directly into the host cell. After entering the nucleus, YfiD attaches to the ADP-ribosyl transferase domain of PARP1, preventing it from connecting the PAR chains to other proteins. This mechanism suppresses PAR accumulation in a manner similar to that of pharmacological PARP1 inhibitors. YfiD binding specifically disrupts the helical domain structure of PARP1, releasing its inhibitory effect on the ADP-ribosyl transferase domain. Through PARP1 inhibition, YfiD diminished macrophage inflammatory responses and cell death, thereby enhancing *E. piscicida* colonization and virulence *in vivo*.

Until recently, most immunotherapies for bacterial infections have focused on drug-resistant *Mycobacterium tuberculosis*, one of the deadliest pathogens that may have killed 1.8 million people in 2020 alone. Cyclooxygenase inhibitors (COXis), such as ibuprofen and celecoxib, are commonly used to relieve tuberculosis-associated symptoms. Previous mouse studies on acute tuberculosis infection suggested that COXis has potential as a host-directed therapy. However, a recent study revealed that treating mice with COXis impaired their ability to control tuberculosis infection in models exposed to respiratory viruses.[118] The negative impact on infection control appears to be linked to the effects on the type 1 T helper cell immune response. Mice receiving COXis showed significantly less CD4+ T cell differentiation into Th1 cells, which is important for fighting tuberculosis. If similar effects are observed in clinical trials, this finding could substantially change global public health strategies and recommendations regarding the use of COXis for patients with tuberculosis.

Moreover, a deeper understanding of the roles of virulence gene expression, immune signaling pathways, and host defense mechanisms is essential for designing effective immunotherapeutic strategies. For instance, current research highlights unresolved questions about how *P. aeruginosa* modulates host immunity.[119] Nonetheless, systems biology approaches, such as transcriptomics, proteomics, and metabolomics, can provide valuable insights into these complex interactions. For instance, an inducible CRISPR interference (CRISPRi) system was developed for *S. pneumoniae* to enable genome-wide fitness screening during both *in vitro* growth and *in vivo* infection.[120] This CRISPRi-seq approach identified genes required explicitly for pneumococcal virulence and replication in a mouse model of influenza coinfection, distinct from those needed for *in vitro* growth. This approach established CRISPRi-seq as a transformative tool for investigating pathogenic mechanisms through genome-wide interrogation during active host infection. By using CRISPRi-seq, future research can screen for immune-evasive mechanisms and identify ideal antigens for therapeutic vaccines, aiming to tip the balance in favor of the host immune response. Hence, to combat bacterial immune resistance, it is critical to better understand bacterial immune evasion mechanisms, including capsule formation, antigenic variation, phagocytosis inhibition, toxin production, and escape from intracellular killing.

Furthermore, comprehending the intricate interplay between the host immune system and the microbiota, the assemblage of bacteria residing within and on the human body, is paramount, as the microbiota plays a pivotal role in instructing and adjusting the immune system. Disruptions in the microbial community composition, known as dysbiosis, can lead to altered immune responses and increased susceptibility to infections. Emerging data have argued that the microbiome balance promotes vaccine efficacy, which deserves consideration. Therefore, studying the interplay among the immune system, microbiota, and bacterial immune resistance is essential for developing effective immunotherapies. Future platforms integrating immunomodulators and microbiome optimization could potentially counter immune evasion.

Advancing Immunotherapy Platforms: Next-Generation Vaccines and Adaptive Cell Therapies. Vaccines represent a pivotal tool, although conventional platforms may lack the breadth or longevity required to eradicate highly adaptive pathogens. Promising alternatives are now garnering a great deal of attention. For example, self-amplifying mRNA (saRNA) vaccines offer one path forward, achieving protective immunity with low dosing amenable to surge production during outbreaks. Notably, a novel SARS-CoV-2 mRNA vaccine encoding a stabilized prefusion spike protein recently demonstrated remarkable efficacy, inducing robust T cell and antibody responses with just a 1 μg dose in nonhuman primates while providing complete protection against mucosal challenge.[121,122] The inherent immunogenicity of this agent, coupled with its scalable and straightforward production, suggests its broad applicability to other infectious threats, including bacterial infections.

Furthermore, multivalent vaccines may avert resistance by curtailing the selective evolutionary pressure on individual antigens. As a proof of concept, a polyvalent group B *Streptococcus* vaccine encoding 10 surface proteins was developed, inducing functional antibodies against all targets and providing protection against lethal challenge in mice.[123] The

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success of this strategy indicates the potential of using multicomponent regimens against other versatile pathogens.

While vaccines marshal population-level protection, cellular therapies offer precise ways to reprogram individual immunity. Chimeric antigen receptor T cells (CAR-T) genetically modified to target microbial epitopes have shown early promise against multidrug-resistant *Mycobacterium tuberculosis*,[79] but they require refinement for safe, scalable use against other bacterial infections. Alternative cellular reprogramming through epigenetic manipulation can circumvent the challenges of CAR-T. For instance, research has shown that macrophages differentiated from human induced pluripotent stem cells can be developmentally "trained" to acquire enhanced antibacterial capabilities.[124] Further elucidation of the mechanisms underlying such reprogramming could unlock new cellular therapies.

Adverse Effects and Safety Considerations. Although immunotherapy can successfully target bacterial cells, it can also negatively impact the body's healthy tissues if not carefully administered. This unintentional reaction, classified as immune-related adverse events (irAEs), occurs when treatment overactivates the immune system against uninfected host cells. One of the most significant concerns with immunotherapies is the potential for excessive immune stimulation, leading to a cytokine storm. This syndrome is characterized by the overproduction of pro-inflammatory cytokines, including IL-1, IL-6, IFN-γ, and TNF-α, resulting in systemic inflammation and organ damage. In cancer immunotherapy, cytokine release syndrome (CRS) is a well-established complication associated with CAR-T cell therapy, immune checkpoint inhibitors, and bispecific T cell engagers. The pathophysiology of CRS involves overstimulation of the immune system, leading to excessive cytokine secretion. If left unchecked, this can result in multiorgan failure and death.

Another concern is the potential for off-target effects, where the immunotherapy inadvertently targets healthy tissues expressing the same or similar antigens as the bacterial pathogen. This can lead to irAEs, including autoimmunity and tissue damage. Immune checkpoint inhibitors (ICIs), for instance, augment T cell function, potentially resulting in irAEs in a notable number of patients. These irAEs may be present in different organs, such as the gastrointestinal tract, liver, lungs, and endocrine glands.

While less characterized in bacterial infections, the potential for a similar phenomenon exists when using immunomodulatory approaches to combat bacterial pathogens. Preclinical studies in animal models have yielded valuable insights into the safety and efficacy of various immunotherapeutic approaches for bacterial infections. Researchers have investigated the use of antimicrobial defense peptides, also known as immunomodulatory peptides, to amplify the elimination of MDR bacterial pathogens and mitigate tissue damage resulting from inflammation. These peptides, including synthetic variants called immunomodulatory regulators (IDRs), have shown promise in protecting against infections without direct antimicrobial action. For example, the synthetic host defense peptide IDR-1002 has been evaluated as a potential therapeutic against *P. aeruginosa* lung infections.[125] The results demonstrate that IDR-1002 does not induce significant proinflammatory responses but can effectively limit the inflammatory cytokine and chemokine production triggered by *P. aeruginosa* or its components *in vitro*. In a chronic mouse lung infection model using *P. aeruginosa* embedded in alginate, IDR-1002 treatment did not reduce the bacterial load but significantly decreased lung inflammation, as evidenced by reduced IL-6 levels and decreased alveolar macrophage infiltration. These findings suggest that IDR-1002 may be a promising adjunct therapy for combating the excessive inflammation associated with chronic *P. aeruginosa* lung infections, such as those observed in cystic fibrosis, without directly targeting the bacteria and risking the development of antimicrobial resistance.

In addition, multiple strategies can be implemented to mitigate potential adverse effects and bolster the safety profile of immunotherapy in the context of bacterial infections. For example, conducting a comprehensive risk–benefit analysis is paramount before contemplating immunotherapy for such diseases. This analysis should consider the availability of alternative treatments, the severity and prognosis of the infection, and the overall health condition of the patient. Immunotherapy may be a valuable option when antibiotic resistance limits treatment options. In cases where bacteria have evolved mechanisms to evade the immune response and persist despite antibiotic therapy, immunotherapy may help restore immune control. In addition, immunotherapy could provide an alternative approach to prevent or treat infection with bacterial pathogens, where effective vaccines are not available. However, immunotherapy may be less effective or harmful in patients with pre-existing immune deficiencies or other conditions impairing the immune function. Similarly, patients with a history of autoimmune disorders or other conditions that predispose them to immune hyperactivation may be at a higher risk of developing severe irAEs from immunotherapy.

Besides thorough screening of patients before immunotherapy, developing biomarkers to predict the risk of cytokine storm and other irAEs is essential for personalized risk assessment and management. These biomarkers could include the levels of specific cytokines, immune cell subsets, and genetic markers. Moreover, using immunomodulatory agents, such as corticosteroids or anticytokine antibodies, may help prevent or mitigate excessive immune activation. However, these agents can also suppress the immune response and increase the risk of secondary infections, so their use must be carefully balanced. Another strategy known as suicide gene therapy involves introducing a gene into immune cells that can be activated to induce cell death, providing a mechanism to eliminate these cells if they cause severe irAEs. This approach has shown promise in cancer immunotherapy and could be adapted for use in bacterial infections.

Targeted Delivery, Monitoring, and Optimization Strategies. To address immune-related irAEs, it is imperative to investigate targeted drug delivery systems. Strategies such as nanoparticle-based delivery systems, conjugated antibodies, and localized delivery techniques are currently being explored to optimize the effectiveness of immunotherapies against bacterial infections.

Nanoparticles (NPs) serve as advantageous delivery platforms for immunotherapeutic agents. They can shield drugs from degradation, improve their absorption by immune cells, and enable precise delivery to the infection site. Cascade-targeted nanoplatforms are designed to target both macrophages and intracellular bacteria. They are fabricated by encapsulating therapeutic agents into nanoparticles coated with phosphatidylserine (PS), which simulates an "eat me" signal to macrophages. After macrophage uptake, the nanoplatforms release their payloads in response to the acidic environment of the phagolysosomes. Another strategy involves engineering the nanoplatforms to stimulate the production of ROS and NO in infected macrophages, thereby enhancing their antibacterial

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capacity. This can be achieved by incorporating agents such as *N*,*N*′-bisacryloylcystamine (BAC), which depletes glutathione (GSH) and allows the generation of ROS. Various types of nanocarriers, such as liposomes, polymeric nanoparticles, dendrimers, micelles, and inorganic nanoparticles, have been explored for the targeted delivery of immunotherapies. Biomimetic nanoparticles that mimic natural immune cells, exosomes, or pathogen structures can enhance the targeting precision, circulation longevity, and cellular uptake efficiency. For instance, cell membrane protein-decorated nanoparticles demonstrated significantly improved cellular binding and internalization, with even partial protein coatings remaining sufficient for phagocyte uptake. To mention a few, a novel nanosystem formulation, termed M33-NS, was recently developed by conjugating the SET-M33 peptide to single-chain dextran nanoparticles.[126] Pulmonary delivery of M33-NS to a mouse model of *P. aeruginosa* respiratory infection revealed the preferential accumulation of nanoparticles in the lungs. Therefore, the M33-NS pulmonary drug delivery system shows promise to optimize the treatment of drug-resistant bacterial lung infections by achieving high local drug concentrations while limiting systemic exposure. Another strategy is to engineer carriers that exploit microbial iron acquisition mechanisms that are indispensable for virulence. For instance, a novel nanocarrier system was recently developed using saponins from *Glycyrrhiza glabra* (liquorice) encapsulated in ferritin nanoparticles (nano-saponin) for bacterial pneumonia treatment.[127] Compared with the untreated pneumonia group, nanosaponin significantly decreased the serum IL-4 levels, lung TNF-α gene expression, and pulmonary COX-2 protein expression. While free saponin showed some effect, nanosaponin performed better, potentially due to targeted nanoparticle delivery. The ferritin nanoparticle delivery platform presents opportunities for designing targeted drug delivery systems. In addition to passive targeting, stimuli-responsive nanocarriers that release immunotherapies upon detecting specific microbial signatures or environmental cues, such as pH, temperature, or enzymes, can further enhance the therapeutic efficacy of immunotherapies by ensuring that the active agents are released only at the target site. Integrating molecular biosensing, feedback control, and triggered release mechanisms into nanomedicine platforms represents an exciting frontier for precision immunotherapy administration.

Conjugated antibodies, commonly termed antibody−drug conjugates (ADCs), are antibodies that are chemically linked to a drug or other therapeutic agent. This approach allows for the targeted delivery of the therapeutic agent to cells that express the antibody-recognized antigen. Conjugated antibodies function by specifically binding to an antigen on the surface of a target cell. Once bound, the ADC is internalized into the cell, where the drug is released, leading to cell death or the inhibition of cell growth. Antibody−antibiotic conjugates enhance targeted drug delivery to bacterial infection sites while minimizing systemic side effects. Recent advances in ADC design have enabled extracellular drug release, overcome the traditional limitations of intracellular antibiotic activation, and improved the efficacy against biofilms. For example, ADCs delivering the potent antimicrobial mitomycin C have been engineered to target *S. aureus*, offering a promising strategy against resistant infections[128] The ADCs were designed to release the drug at the bacterial cell surface without requiring cell entry. They could bind to *S. aureus* in both the planktonic and biofilm states. Importantly, the *S. aureus*-targeted ADCs showed superior antimicrobial activity compared with nontargeted ADCs both *in vitro* and in a mouse model of implant-associated osteomyelitis.

Despite the significant promise of conjugated antibodies in enhancing the treatment of bacterial infections, creating high-affinity antibodies that effectively bind to specific antigens on bacterial or immune cells can pose a formidable challenge. The existence of specific bacterial proteins, such as staphylococcal protein A (SpA), can bind to antibodies and hinder their optimal functionality. Consequently, altering the Fc region of antibodies can boost their capacity to mobilize immune responses and improve their half-life, which is crucial for effective bacterial targeting. For example, engineered variants of the anti-*S. aureus* antibodies 3F6-hIgG1 and Tefi were developed by introducing specific amino acid substitutions in the Fc region to disrupt protein A (SpA) binding[129] This restored the antibodies' ability to recruit complement C1q and promote opsonophagocytic killing of *S. aureus*. In addition, the Fc-engineered antibodies improved therapeutic efficacy against MRSA infection in mouse models compared to the unmodified antibodies.

Also, localized delivery techniques using biomaterials for administering therapeutic substances directly to bacterial infection sites offer significant advantages over systemic treatments. These methods can achieve drug concentrations that surpass the minimum inhibitory concentration (MIC) for extended periods, enhancing the therapeutic effect. By targeting the infection site directly, these systems minimize systemic exposure and associated side effects, which is particularly beneficial in chronic conditions like osteomyelitis.[130] Local delivery can also improve outcomes in infections involving biofilms, such as those in prosthetic joint infections, by maintaining high local antibiotic concentrations. Among the biomaterials, biodegradable polymers offer a promising alternative, eliminating the need for removal and providing sustained drug release. Hydrogels and calcium sulfate allow for adjustable drug release profiles and are being explored for their compatibility with various antibiotics. However, their clinical use is still limited. Managing the initial burst release of drugs and potential tissue toxicity remains a challenge, necessitating further research to optimize the release kinetics.

Monitoring the therapeutic response remains essential for personalized management; however, serial sampling poses infection risks and lacks spatiotemporal resolution. Emerging alternatives such as rapid noninvasive diagnostics and breath analysis are promising tools for real-time disease monitoring via volatile organic compound (VOC) detection. For instance, unique breath VOC signatures linked to *Pseudomonas aeruginosa* pneumonia were identified in a mouse model, correlating with pathogen burden, inflammatory state, and treatment response.[131] As breath collection poses a minimal risk to vulnerable patients, developments enabling the integration of such data into dynamic treatment algorithms are attractive prospects. Nonetheless, an alternative monitoring approach seeking direct real-time visualization at the infection sites holds some promise. As a proof of concept, Chagnon and colleagues[132] developed microscale endoscope devices compatible with *in vivo* lung deployment in mice for direct fluorescent imaging of acute lung injury. Implanted devices incorporating both diagnostic and therapeutic modules responsive to molecular signals represent an on-the-horizon technology for transforming high-fidelity personalized tracking and treatment response management.

Clinical Integration. Transitioning promising preclinical findings to practical solutions requires robust clinical evaluation

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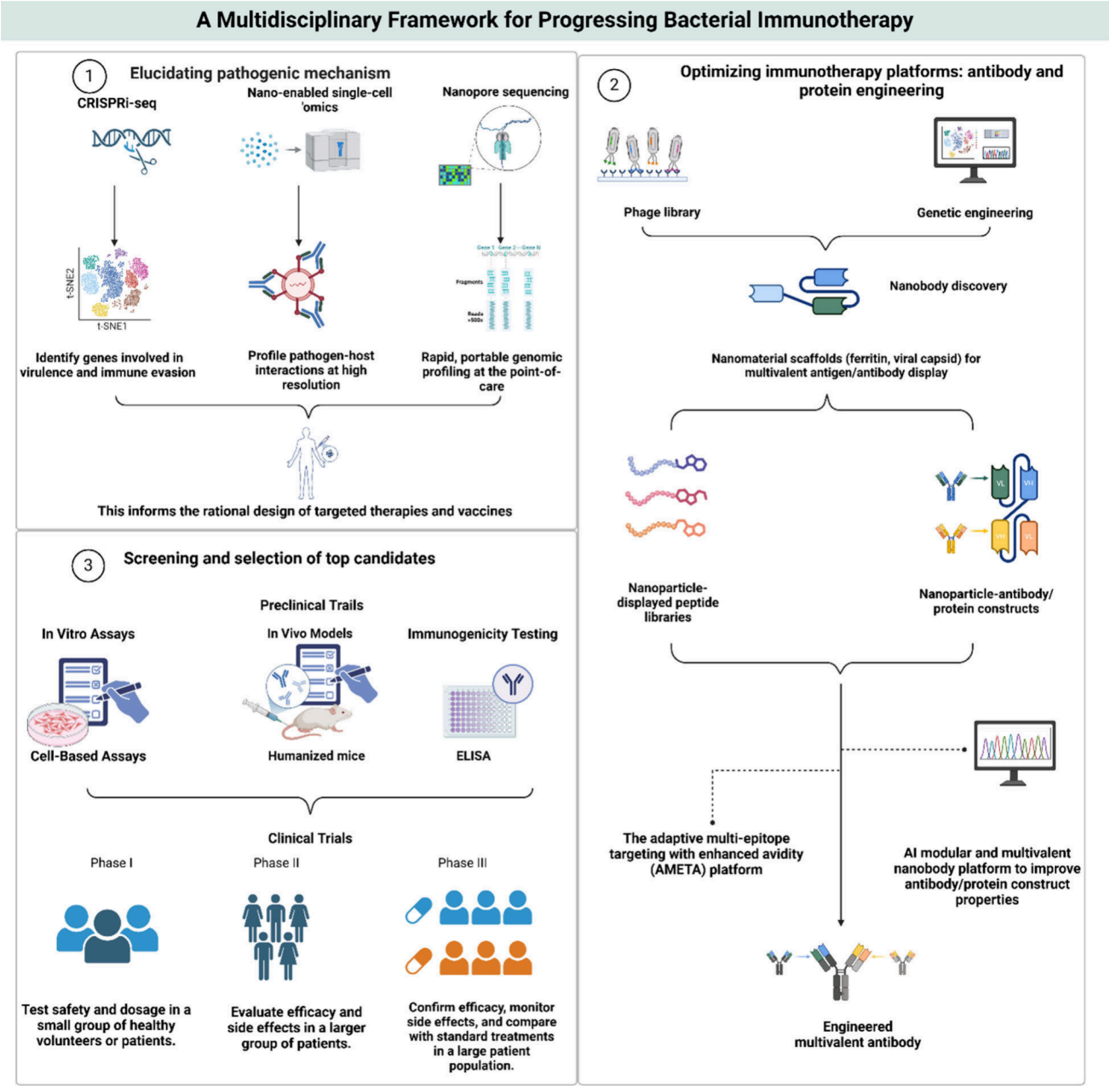

Figure 5. Proposed multidisciplinary framework for the advancement of bacterial immunotherapy. In discovery efforts, integrated approaches are applied to identify agents that selectively recognize pathogens. Techniques such as CRISPRi-seq can identify genes involved in virulence and immune evasion. Nanoenabled single-cell omics for profiling pathogen-host interactions. This information informs the rational design of targeted therapies and vaccines.[1] Antibody and protein engineering using bioinformatics and computational modeling aids in improving construct stability, production, and other properties.[2] Screening and selection of potential antibacterial candidates, which are then tested in preclinical/clinical trials.[3]

frameworks that incorporate an evolving understanding of immunotherapeutic complexity. A recent study on leukemia immunotherapy explored how the gut microbiome influences clinical outcomes after anti-CD19 CAR-T cell therapy.[133] The study revealed that receiving certain antibiotics, such as meropenem, imipenem/cilastatin, and piperacillin/tazobactam, within 4 weeks of CAR-T cell therapy was linked to poorer survival rates and heightened neurotoxicity. Compared with healthy controls, the microbiomes of CAR-T cell patients exhibited alterations in microbial composition and diversity.

Similar approaches may optimize bacterial immunotherapy when coupled with longitudinal omics at multiple time points. Advanced analytics is now empowering such approaches. For instance, machine learning models integrating multiomics data and clinical records from over 5,000 tuberculosis (TB) patients across 10 high-burden countries were developed to identify the key factors affecting TB treatment outcomes.[134] The study demonstrated enhanced accuracy in outcome prediction when using integrated models compared with individual modalities. Interestingly, the analysis uncovered correlations between specific drug regimens and treatment success or failure. For instance, the combination of clofazimine, cycloserine, linezolid, levofloxacin, and bedaquiline was associated with successful treatment for multidrug-resistant extensively drug-resistant TB. In contrast, linezolid, bedaquiline, moxifloxacin, and clofazimine were linked to treatment failure. Furthermore, the drug

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combinations predicted by the INDIGO algorithm to be synergistic outperformed those predicted to be antagonistic. This comprehensive modeling approach identified a prioritized set of diagnostic and treatment factors that can assist in tailoring personalized clinical care for TB patients.

Also, novel trial formats facilitate real-world evidence gathering. Master protocols enable streamlined and simultaneous evaluation of combination regimens through independent yet coordinated substudies. TB research and innovation are among the three pillars of the WHO's End TB Strategy. This approach has been adopted by conducting globally integrated subtrials of host-directed therapies and vaccines as adjuncts to standard treatment. Although promising, the evaluation of immunotherapies requires a global healthcare resource investment. Thus, demonstrating cost-effectiveness and feasible implementation warrants our attention. Recent economic analyses have evaluated the potential impact of scaling up an experimental tuberculosis vaccine in low- and middle-income countries (LMICs), estimating its value in reducing disease burden while offering a framework to assess societal returns on innovation investments.[135] International consortia now play a crucial role in integrating cross-disciplinary expertise, data sharing, and coordinated multisite trials to optimize translation. For instance, the *Global Roadmap for Research and Development of Tuberculosis Vaccines* outlines global efforts to validate and integrate new drug regimens informed by biomarkers. Similar initiatives against other drug-resistant threats will accelerate progress through open collaboration.

CONCLUSION AND OUTLOOK

The unique mechanisms and benefits of immunotherapy make it a compelling alternative to antibiotic bacterial infection treatment. As research and clinical experience in this field expand, enhancing current strategies through a multidisciplinary approach integrating engineering, synthetic biology, computational analytics, and *in vitro* validation can yield more durable protection (Figure 5). For instance, a species-specific nanobody targeting *Acinetobacter baumannii* was developed through an integrated phage display and bioinformatics approach.[136] This study exemplifies how combining synthetic biology, sequencing, computational analysis, and experimentation can successfully discover pathogen-targeting agents for diagnostic and therapeutic purposes.

Moreover, antibody engineering using techniques such as multivalent displays can enhance the potency of antibody immunotherapy. A recent breakthrough study developed an innovative multivalent antibody platform called adaptive multiepitope targeting with enhanced advection (AMETA) for superior antiviral potency against rapidly evolving pathogens. AMETA utilizes a human IgM scaffold with over 20 attached bispecific nanobodies.[137] This modular design allows targeting of multiple conserved neutralizing epitopes on the viral surface. Remarkably, through avid, multivalent binding, AMETA constructs boost antiviral activity by more than a million-fold compared with monomeric nanobodies. This modular platform could be expanded to create multipathogen targeting "polyclinics" against drug-resistant bacteria, although challenges persist in optimizing construct stability, manufacturability, and pharmacokinetics.

Integrating computational tools with ML or AI-based prediction, incorporating structural modeling, molecular dynamics, and informed library design, shows promise in overcoming these barriers and may further optimize such constructs. AI-powered platforms can simulate antibody−antigen interactions, enabling researchers to design and evaluate antibodies virtually before advancing to the experimental stages. These machine learning models facilitate the swift creation of antibody candidates *in silico*, reducing the development time by about 60% and the costs by 50% compared to traditional approaches.[138] An updated version of the AlphaFold system (AlphaFold 3) has been released, incorporating improvements to the structural prediction algorithm.[139] Although AI algorithms can predict the structure and binding affinity of antibodies, reducing the need for extensive experimental screening, it is important to acknowledge that even with AlphaFold 3 AI-based predictions of paratope−epitope interactions may still lack accuracy. Consequently, the experimental validation of the epitopes remains a critical step in the successful selection of neutralizing antibody leads.

Furthermore, multitarget immunotherapies, engineered bacteriophages, and CRISPR-based antimicrobials offer promising avenues for future research. Multitarget immunotherapies can simultaneously target multiple bacterial virulence factors, enhance bacterial clearance, and modulate the host immune response. As a proof of concept, chimeric proteins were engineered by fusing *Moraxella*-binding domains to immunoglobulin Fc regions, demonstrating a novel targeting strategy.[140] These fusion proteins enhanced bacterial killing through complement activation and displacement of complement inhibitors. This approach could be adapted to target other bacterial pathogens and enhance their clearance through enhanced opsonization, phagocytosis, and complement-mediated killing. Engineered bacteriophages offer a versatile platform for the targeted delivery of antibacterial and immunomodulatory agents. Genetic engineering enables the alteration of phages to augment their therapeutic potential and expand their applications. One promising strategy involves engineering phages to express anti-CRISPR (Acr) proteins, which can inhibit the activity of bacterial CRISPR-Cas systems. For example, engineered bacteriophages carrying Acr genes have demonstrated significant efficacy against MDR *P. aeruginosa* infections.[141] Also, a novel phage engineering system (SpyPhage) was developed by incorporating SpyTag moieties on phage capsid heads, enabling efficient postsynthetic modification through covalent conjugation with SpyCatcher-fused proteins.[142] This innovative system enables the swift deployment of customized engineered variants essential for combating mutant bacteria that may arise during a pandemic. Moreover, the SpyPhage system offers a streamlined production process for generating multiple phenotypically diverse phages from a single underlying genotype.

Phages can be engineered to deliver immunomodulatory molecules, such as cytokines, chemokines, or antimicrobial peptides (AMPs), directly to the site of infection. Immunomodulatory peptides demonstrate significant potential for both enhancing pathogen clearance and reducing infection-associated tissue damage.[143] However, engineering phages to express and deliver these peptides could provide a targeted approach to modulating the immune response and promoting bacterial clearance. Several bacterial pathogens, including *E. coli* and *Salmonella*, can induce intracellular diseases by infiltrating host cells and evading the immune system. Engineered phages equipped with cell-penetrating peptides (CPPs) can significantly boost their internalization efficiency within host cells, enhancing their efficacy against intracellular bacterial infections. For instance, research has demonstrated that the EGF-

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conjugated phage K1F exhibits enhanced cellular internalization in human cell lines, effectively clearing intracellular *E. coli* K1 infections.[144]

CRISPR-Cas systems offer a powerful tool for precisely targeting bacterial species and antibiotic resistance genes in a sequence-specific manner. They can induce antimicrobial effects by deactivating chromosomal genes or eliminating plasmids harboring antibiotic resistance genes. This precision enables the targeted management of microbial populations, potentially treating complex infections involving multiple microorganisms or manipulating microbial communities. For instance, conjugative delivery of CRISPR-Cas9 has been demonstrated to be effective in targeted elimination of MDR *Enterococcus faecalis*.[145] The potential of utilizing endogenous CRISPR nucleases for genome modification and developing CRISPR-based antimicrobial agents is being explored. Advancements in genome editing with endogenous CRISPR-Cas systems are poised to streamline procedures and enhance editing efficacy in prokaryotic cells. Endogenous CRISPR-Cas systems offer an alternative to the more commonly used Cas9, Cas12, and Cas13, which are characterized by their substantial size, posing manipulation challenges, and potential to cause cell toxicity.

Although these innovative therapeutic approaches hold significant promise, several challenges must be addressed to enable the clinical translation and widespread implementation. These challenges include regulatory hurdles, safety concerns, and bacterial resistance mechanisms.[146] Addressing these issues through comprehensive preclinical and clinical evaluations is imperative to ensuring the extensive acceptance of phage therapy. Implementing strategies to combat resistance, such as the use of phage cocktails and enhancement of CRISPR-Cas systems targeting multiple sites, is vital for the sustained efficacy of these interventions. Therefore, a multifaceted approach that combines these strategies (Figure 5) will be essential for effectively managing bacterial infections in the postantibiotic era.

■ ASSOCIATED CONTENT

Data Availability Statement

All supporting data are presented in the manuscript.

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Author Contributions

A.O. conceptualized and drafted the manuscript. A-H.O. drafted the manuscript. C.K.O.D conceptualized, revised and supervised the work. All authors have read and approved the final version.

Notes

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■ ABBREVIATIONS

mAbs, monoclonal antibodies; KBMA, killed but metabolically active; GBS, group B *Streptococcus*; MRSA, methicillin-resistant *Staphylococcus aureus*; OprF, porin F; OprI, lipoprotein I; TcpA, toxin-coregulated pilus subunit A; CtxB, cholera toxin subunit B; OmpW, outer membrane protein W; PAD, fusion construct of the immunodominant regions of PspA and PhtD; ΔaroA, deletion of the aroA gene; PcrV, type III secretion system protein; OprF, outer membrane protein F; OprI, outer membrane lipoprotein I; PBP2a, penicillin-binding protein 2a; LukAB, leukocidin AB; SpA, staphylococcal protein A; AuNP, gold nanoparticle; Ab-NP, *A. baumannii* nanoparticle vaccine; OMVs, outer membrane vesicles; CAR, chimeric antigen receptor; CIK, cytokine-induced killer; MDR-TB, multidrug-resistant tuberculosis; CASP11, caspase-11; M. tb, *Mycobacterium tuberculosis*; IONPs, iron oxide nanoparticles; ROS, reactive oxygen species; NO, nitric oxide; ALPK1, α-kinase 1; IgY, avian immunoglobulin Y; ADAs, antidrug antibodies; ML, machine learning; AI, artificial intelligence; CRS, cytokine release syndrome; irAEs, immune-related adverse events; PT3X, pentraxin 3; CRAMP, cathelicidin-related antimicrobial peptide; MSCs, mesenchymal stem cells; HCECs, human corneal epithelial cells; Ch25h, cholesterol 25-hydroxylase; IDR, innate defense regulator; ATRA, all-trans retinoic acid; Q-VD-OPH, quinoline−valine−aspartic acid−difluorophenoxymethyl ketone; ABL/PI5P, apoptotic body-like liposomes loaded with phosphatidylinositol 5-phosphate; MDR-KP, multidrug-resistant *Klebsiella pneumoniae*; COXis, cyclooxygenase inhibitors; saRNA, self-amplifying RNA; AB, *Acinetobacter baumannii*; CXCL, C-X-C motif chemokine ligand; ARDS, acute respiratory distress syndrome; ADC, antibody−drug conjugate; BAC, *N*,*N*′-bisacryloylcystamine; GSH, glutathione; PMMA, polymethylmethacrylate; VOC, volatile organic compound; IVIG, intravenous immunoglobulin; AGP, aminoalkyl glucosaminide 4-phosphate; CRX-527, synthetic TLR4 agonist; TLR, toll-like receptor; TNF-α, tumor necrosis factor-α; IL, interleukin; QALYs, quality-adjusted life years; HTA, health technology assessment

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