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The Therapeutic Pipeline for *Pseudomonas aeruginosa* Infections

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ABSTRACT: *Pseudomonas aeruginosa* is a Gram-negative opportunistic pathogen, designated by the World Health Organization as a critical priority for development of new therapeutics due to high levels of intrinsic and acquired antibiotic resistance. Other challenges include its versatility (it can persist in the environment and most strains are capable of causing disease in compromised hosts), robust efflux mechanisms that limit drug penetration, and the propensity to form antimicrobial-tolerant biofilms. Novel therapeutics in development to prevent or treat *P. aeruginosa* infections include vaccines, biologics such as antimicrobial peptides and therapeutic antibodies, virulence inhibitors, antimicrobials with novel targets, antibody−drug conjugates, resistance inhibitor−antibiotic or antibiotic−potentiator combinations, and bacteriophages or phage-derived lysins.

The Gram-negative opportunistic pathogen *Pseudomonas aeruginosa* infects compromised hosts, particularly people with cystic fibrosis (CF) and bronchiectasis where it causes chronic lung infections.[1] Its propensity to form recalcitrant antibiotic- and disinfectant-tolerant biofilms make it a scourge in hospitals, especially intensive care and burn units, where it colonizes plumbing fixtures and medical devices such as urinary catheters and endotracheal tubes. It can cause a range of nuisance infections including eye (associated with contact lenses) and ear infections that can escalate if untreated.[2] *P. aeruginosa* is considered a serious threat by the U.S. Centers for Disease Control and is among the World Health Organization's critical priorities for development of new therapeutic strategies.[3]

WHY IS TREATMENT OF *P. AERUGINOSA* INFECTIONS PROBLEMATIC?

It Is Intrinsically Resistant to Many Antibiotics. *P. aeruginosa* combines an outer membrane of limited permeability with an array of efflux pumps to efficiently limit the access of antibiotics to their targets, including antibiotics to which it has never been exposed.[4−6] It readily acquires mutations in efflux pump repressors, leading to constitutive expression.[7,8] It encodes an inducible AmpC β-lactamase that degrades multiple β-lactam antibiotics and can accumulate porin mutations that block carbapenem import.[9] It can carry plasmids encoding, among other resistance elements, metallo-β-lactamases and carbapenemases. These features mean that presumptive *P. aeruginosa* infections require use of second or third line drugs, creating the potential for further resistance development. Resistance rates are higher in resource-limited settings where less effective drugs are used to treat *P. aeruginosa* infections.

It Has a Large Genome Encoding Extensive Regulatory Capacity and Metabolic Flexibility. Humans are only one of many hosts, including fungi, plants, worms, insects, and higher eukaryotes, that can be infected by *P. aeruginosa*.[10] It can cause both acute and chronic infections, adjusting its metabolism and virulence factor repertoire expression accordingly.[11] It can grow aerobically or anaerobically if suitable electron acceptors are available.[12] It is a formidable competitor against other microbes in polymicrobial infections, producing multiple antibacterial effectors.[13] Finally, phenotypic microheterogeneity in genetically identical populations is common, providing additional resiliency against unexpected environmental insults.[14]

It Is a Prolific Biofilm Former, a Capacity Enhanced by Exposure to Subinhibitory Concentrations of Antibiotics. *P. aeruginosa* is a popular model organism for the study of bacterial biofilm development. It forms antimicrobial- and disinfectant-tolerant communities on biotic and abiotic surfaces, under a variety of nutrient conditions. Using swimming, swarming, and twitching motilities, it rapidly spreads on surfaces and will actively migrate against fluid flow to colonize regions inaccessible to other species.[15] The biofilm environment promotes development of small colony variants and persister cells that can withstand high concentrations of antibiotics.[16,17] Effective suppression of the growth of polymicrobial biofilms that include *P. aeruginosa* in the lungs of CF and bronchiectasis patients requires high local drug concentrations delivered by aerosol, but penetration into deeper sites can be limited. The resulting exposure of *P. aeruginosa* to subinhibitory concentrations of antibiotics, from multiple classes, stimulates biofilm formation by as-yet unknown mechanisms,[18,19] meaning that suboptimal treatment regimens could perversely worsen infection.

***P. aeruginosa* Infections Have Environmental Reservoirs.** Prevention of disease through prophylactic strategies such as vaccination is always preferable to treatment that may fail. However, aside from high-risk populations such as those with CF, where a few highly transmissible epidemic strains have been identified,[20,21] it could be tough to make a clear cost-benefit case for widespread *P. aeruginosa* vaccine prophylaxis. The strains that cause disease are frequently acquired from environmental reservoirs[22−24] where the organism persists on moist surfaces, so therapeutics must be designed to target

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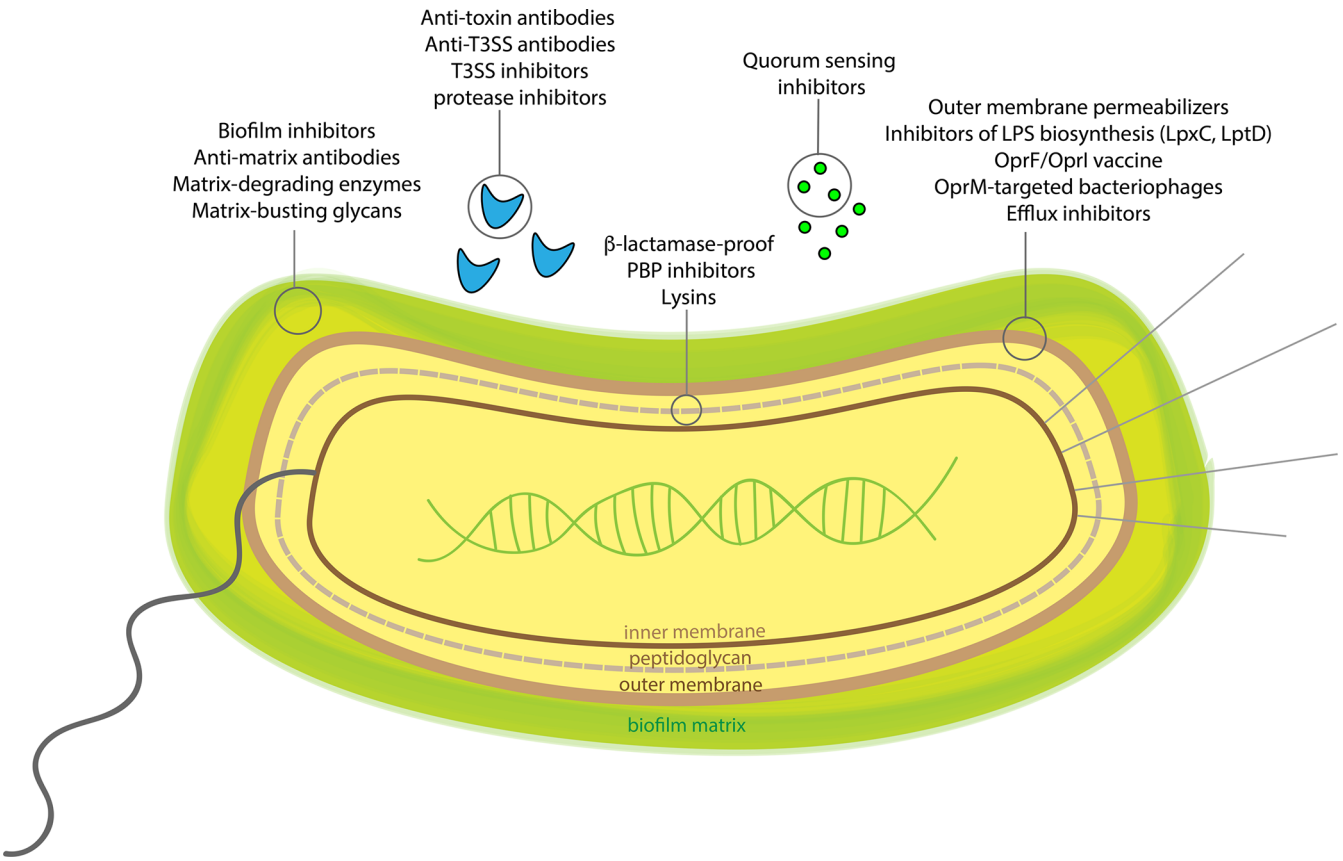

Figure 1. Examples of novel therapeutics for *Pseudomonas aeruginosa*.

universal elements conserved among strains with diverse sets of virulence factors and antibiotic resistance profiles.

WHAT IS IN THE ANTI-*PSEUDOMONAS* PIPELINE?

With *P. aeruginosa* close to the top of most "bad bugs" lists, it is in the crosshairs of multiple small molecule discovery and development programs aimed at both old and new targets. Other types of novel therapeutics and prophylactics are also under investigation (Figure 1). Grassroots discovery efforts have been amplified by recent strategic investment programs, including the Innovative Medicines Initiative (IMI), the Joint Programming Initiative on Antimicrobial Resistance (JPIAMR), and the Combating Antibiotic Resistant Bacteria Biopharmaceutical Accelerator (CARB-X).[25−27] A sample of what is in the pipeline is presented in Table 1 and below. For more detailed information, readers are directed to recent reviews and commentaries.[28−36]

Novel Antibiotics, Antibiotic Combinations, and Potentiators. Peptidoglycan biosynthesis remains among the best-validated and privileged targets for antimicrobials. Many discovery efforts are aimed at designing molecules that block, or are resistant to, β-lactamases, especially metallo-β-lactamases and carbapenemases. Two new antibiotic−inhibitor combinations, ceftazidime−avibactam (Allergan, https://www.allergan.com/home) and ceftolozane−tazobactam (Merck, http://www.merck.com/), were recently launched, and Entasis Therapeutics (http://www.entasistx.com/) is working on new non-β-lactam penicillin-binding protein (PBP) inhibitors. While these are welcome additions, all address essentially the same step in peptidoglycan synthesis, stem peptide cross-linking. There are many more druggable targets in this pathway under investigation,[37] and new classes of inhibitors are expected to emerge. However, it is sobering to realize that the majority of molecules currently in Phase I−III clinical trials (http://www.pewtrusts.org/en/multimedia/data-visualizations/2014/antibiotics-currently-in-clinical-development) have no activity against *P. aeruginosa*.

The outer membrane of *P. aeruginosa* is an essential structural component and a substantial barrier to antibiotic penetration. The peptide antibiotics colistin and polymyxin disrupt this barrier and are used to treat multidrug-resistant strains but usually as a last resort due to toxicity. The spread of plasmid-borne colistin resistance enzymes such as MCR-1 may also limit the utility of these compounds.[38] Inhibition of lipopolysaccharide (LPS) biosynthetic or transport enzymes by small molecules could kill Gram-negative bacteria and/or potentiate entry of traditional antibiotics. One of the best studied LPS targets is LpxC, essential for lipid A biosynthesis.[39] Achaogen (http://www.achaogen.com/) is engaged in development of LpxC inhibitors with activity against multidrug-resistant strains; however, one such compound, ACHN-975, was recently withdrawn.[40] Polyphore (https://www.polyphor.com/) is developing murepavadin (POLY7080), an inhibitor of LptD, part of the system that transports LPS molecules from the inner to the outer membrane, that is in Phase III trials. Other groups are working on potentiators for antibiotics that would otherwise be unable to cross the outer membrane, including those typically used for Gram-positive infections. One example is the previously approved antiprotozoal drug, pentamidine, now in preclinical studies as an antibiotic adjuvant.[41] Spero Therapeutics (https://sperotherapeutics.com/) was recently awarded significant funding through

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Table 1. Examples of Novel Therapeutics for *Pseudomonas aeruginosa* Infections

type of therapeutic	target(s)	therapeutic	development stage	company or reference
Small Molecules				
antibiotic-siderophore	penicillin-binding proteins, iron uptake	cefiderocol	Phase III	Shionogi[72]
antibiotic	LpxC (lipopolysaccharide biosynthesis)	multiple	Phase I	Achaeogen[40]
peptidomimetic	LptD (outer membrane assembly)	murepavadin	Phase III	Polyphor/Roche[73]
antibiotic potentiator	outer membrane permeability	pentamidine	hit-to-lead	41
Biologics				
antibody	PcrV (T3SS)	h1F3 (humanized mAb)	preclinical	Shionogi
antibody	LPS (serotype O11)	AR-101 (mAb)	Phase II	Aridis
antibody	PcrV (T3SS) and Psl (matrix polysaccharide)	MEDI3902 (bispecific mAb)	Phase II	Medimmune[52,74]
enyzme	Psl and Pel matrix polysaccharides	PslG, PelA	preclinical	53
glycan	biofilms and motility	Oligo-G(uluronic acid)	Phase II	AlgiPharm[55]
Vaccines				
vaccine	OprF/OprI (porins)	IM vaccine	Phase II	58
Bacteriophages or Phage Components				
phage component	peptidoglycan	lysin	hit-to-lead	Contrafect
phage	OprM	phage	preclinical (compassionate use)	69

CARB-X to advance their Gram-negative potentiator lead, SPR741.

Other potentiators include efflux inhibitors.[42,43] These compounds increase intracellular concentrations of traditional antimicrobials when given in combination. One of the best studied examples, phenylalanine−arginine β-naphthylamide, has dual activities as an efflux inhibitor and an outer membrane permeabilizer, which could reduce the development of resistance, but it has not been used clinically due to toxicity.[44] The large number (at least 12 belonging to the resistance-nodulation-division family alone) and structural diversity of efflux pumps in *P. aeruginosa* means it is tough to develop pan-inhibitors, and nothing has yet advanced beyond preclinical optimization. Because some pumps also export quorum sensing molecules (below), efflux inhibitors could have the added bonus of disrupting virulence and biofilm formation.[45] Potentiators do not necessarily have intrinsic antimicrobial activity; thus, antibiotic−potentiator combinations can still be thwarted by target mutations that confer high level resistance to the antimicrobial.

Biologics. This class of therapeutics includes antimicrobial peptides, antibodies, antibody−drug conjugates, and enzymes.[46] Antimicrobial peptides can have multiple activities, killing bacteria directly, potentiating conventional antibiotics, inhibiting and disrupting biofilm formation, and modulating the host immune response.[47,48] Careful tuning of the properties of these therapeutics in terms of charge and hydrophobicity is important to minimize toxicity. EligoChem (https://eligochem.com/) is developing an antimicrobial peptide (ELIGO-3233) with activity against *P. aeruginosa*, while Visterra (http://visterrainc.com/) has a unique take on antimicrobial peptide delivery, coupling their peptide with a cell surface glycan-targeting monoclonal antibody (VIS705). This narrow spectrum approach provides high local concentrations of the antimicrobial peptide at the cell surface. There are also multiple efforts underway to re-engineer older peptide antibiotics such as polymyxins to reduce their toxicity.[49,50]

In addition to increasing local drug concentrations when formulated as conjugates, antibodies can opsonize bacteria to enhance immune killing, synergize with conventional antibiotics, and neutralize toxins. Cidara (https://www.cidara.com/) is developing a novel LPS-binding therapeutic (CD-201) that can kill *P. aeruginosa* directly (including colistin-resistant strains) and recruit innate immune effectors. Medimmune (https://www.medimmune.com/) is in Phase II clinical trials with a bispecific therapeutic antibody (MEDI-3902) that recognizes both the PcrV protein that forms the tip of the *P. aeruginosa* type 3 secretion system (T3SS) needle and the biofilm matrix polysaccharide Psl, involved in immune evasion. The anti-Psl portion directs the antibodies to the cell surface, where the anti-PcrV portion neutralizes the T3SS machinery, inhibiting the export of toxic effectors. In addition to traditional intravenous administration, the potential to immunize with recombinant DNA encoding the bispecific antibody is being explored as a more cost-effective option.[51] Development of another anti-PcrV monoclonal antibody (KB001-A, KaloBios) was discontinued after it failed to meet primary end points in Phase II clinical trials in CF patients. A recent study[52] suggested that antibodies with the most potent *in vitro* neutralization capacity may not provide the best *in vivo* protection, suggesting that a balance of properties should be considered during development.

Enzymes that degrade biofilm matrix polysaccharides[53] are interesting newcomers to the therapeutic arena. These proteins are normally encoded with other enzymes in the polysaccharide biosynthetic pathways and, when applied externally as purified preparations, have the capacity to both inhibit biofilm formation and disperse existing biofilms. They remain functional when attached to surfaces,[54] suggesting they may be useful to inhibit biofilm development on medical devices.

Another biofilm matrix disruptor is the glycan oligo-G (oligomeric alginate fragments enriched in guluronate residues), produced by AlgiPharma (http://algipharma.com/). These short glycans can be administered as an inhaled powder to the lungs of CF patients, where they reduce viscosity in part by chelation of calcium ions and increase the efficacy of traditional antibiotics.[55,56] This therapeutic is most relevant to *P. aeruginosa* lung and sinus infections, where alginate is an important component of the biofilm matrix.

Vaccines. Immunization of select patient populations at high risk of *P. aeruginosa* infection represents an important strategy to prevent infections by drug-resistant strains, but there are currently no licensed vaccines.[57] A parenteral vaccine containing a recombinant antigen fusing the C-terminal portion

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of conserved porin OprF with porin OprI was recently tested in clinical trials in patients with burns or CF and those undergoing mechanical ventilation.[58] In the latter study, the vaccine failed to protect patients from *P. aeruginosa* disease compared to placebo, and there was an increase in invasive infections in the study population, calling this strategy into question.[59] Trials of other *Pseudomonas* component vaccines in CF patients have similarly failed to show benefit.[60] This is an area that requires further research to ensure the best antigens, routes of administration, and patient populations are selected.

Inhibitors of Virulence. The concept that reducing infection by impairing the expression or function of virulence factors might lead to less resistance than use of antibiotics has received increasing traction.[61] *P. aeruginosa* has multiple virulence factors, but the key role of toxins secreted by the T3SS in infections of mammalian hosts means that this machinery is of particular interest as a target. In addition to therapeutic antibodies targeting the PcrV component mentioned above, small molecule T3SS inhibitors are under development by Microbiotix (http://microbiotix.com/). They identified phenoxyacetamides that, on the basis of mutations conferring inhibitor resistance, are likely to target the needle protein PscF.[62,63] These studies also showed that it is possible to develop resistance to antivirulence compounds. Elastase (LasB) is another important toxin secreted by the type 2 secretion system (T2SS) and a primary cause of tissue damage in *P. aeruginosa* infections. Antabio (https://antabio.com/) has received investments from the Wellcome Trust and CARB-X to develop an inhibitor of this enzyme.

Quorum sensing (QS) is important for regulation of virulence and biofilm formation in response to cell density. Inhibitors of QS could decrease pathogenesis and, through their ability to block biofilm formation, act as potentiators of conventional antibiotics. *P. aeruginosa* has multiple interconnected QS systems, including the Las, Rhl, and Pqs systems. Potent inhibitors of all these systems have been identified,[64] but none have yet made it beyond the preclinical stage of development.

Bacteriophages and Phage Lysins. As success stories emerge and regulatory hurdles are met, bacteriophages (phages) are gaining broader acceptance as therapeutics.[65] They have the advantages of narrow spectrum, self-amplification, and the ability to kill even highly antibiotic-resistant strains.[66] Many *P. aeruginosa* phages recognize LPS or type IV pili as receptors, suggesting that escape mutants could be less pathogenic due to loss of expression of those key virulence factors.

Phages could also be used as antibiotic adjuvants.[67] A phage targeting OprM, the outer membrane component of both the MexAB and MexXY efflux systems, killed *P. aeruginosa* and selected for escape mutants with increased susceptibility to antibiotics due to loss of OprM expression.[68,69] When used in conjunction with antibiotics, this phage successfully cleared a chronic multidrug-resistant *P. aeruginosa* biofilm infection of >4 years in duration.

To address concerns that the use of intact phages as therapeutics may lead to lysogeny or influence bacterial evolution in unexpected, possibly negative, ways, some have turned to using phage-derived lysins, components that cause rapid cell lysis.[70] Some lysins have the capacity to kill nongrowing persisters and biofilm-embedded cells, and they are synergistic with conventional antibiotics. After developing a lysin therapeutic for methicillin-resistant *Staphylococcus aureus* that looks promising in Phase II clinical trials, Contrafect (https://www.contrafect.com/) is now applying their experience to finding lysins with activity against *P. aeruginosa*.

CONCLUSIONS

The state of the pipeline for anti-*Pseudomonas* therapeutics is encouraging, although significant time and effort will be needed before many of these treatments reach the clinic. Importantly, some solutions have the potential to reduce the burden not only of *P. aeruginosa* infections but also of other multidrug-resistant Gram-negative pathogens. Complementing these efforts is the development of rapid diagnostics that will improve our ability to provide more timely, accurate, and targeted interventions. Improved surveillance[71] and stewardship programs are also key to contain the spread of highly resistant strains that could seriously challenge our ability to treat *P. aeruginosa* infections. Continued investment in early stage drug discovery efforts by governments and industry consortia is essential to ensure that novel ideas and approaches continue to flow, since the cost of failure to address this problem will be much higher.

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Notes

The author declares no competing financial interest.

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ABBREVIATIONS

CF, cystic fibrosis; PBP, penicillin-binding protein; LPS, lipopolysaccharide; T2SS, type 2 secretion system; T3SS, type 3 secretion system; QS, quorum sensing

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